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by

John Robert Horsley

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SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

EXPERIMENTAL MEDICINE

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To my wife and children

Lis, Simon and Daniel

ABSTRACT

Methacholine inhalational challenge, a means of assessing nonspecific airways reactivity, is useful for identifying subjects with hyperreactive airways who have episodic symptoms and normal findings on examination. Bronchodilator responsiveness, as an indicator of increased bronchomotor tone, cannot separate out those subjects likely to react to challenge testing from those who will not react. Fifteen patients with episodic cough, wheeze, dyspnea or chest tightness were referred for challenge testing. All had normal physical, radiological and hematological results with normal pulmonary function. Eleven healthy volunteers were also studied, only one of whom had a history suggestive of atopy and none of whom had respiratory symptomatology. All subjects were nonsmokers, the mean age of the 26 subjects being 34 ± 11 years (mean ± 1SD). All had normal initial regional residual volume to regional total lung capacity (RVr/TLCr) results for their age group, measured by ^{133}Xenon radiospirometry using a multi-detector counting system in the sitting position.

Twelve subjects (2 volunteers and 10 referred patients) showed a significant (>20%) fall in FEV_1 after methacholine inhalation, with a PC_{20FEV_1} of 8.7 ± 8.2 mg/ml (mean ± 1 SD). A highly significant increase in RVr/TLCr post-methacholine occurred in lung regions studied at 5, 11, 17, 23 and 29 cm below the top of the lungs ($p<.01$ at 5 cm;

$p<.001$ in all other regions). The maximum increases in RVr/TLCr were observed in the regions 23 and 29 cm below the top of the lungs (50% and 45% increases respectively). Slightly less improvement from the post-methacholine RVr/TLCr measurements were noted in the upper lung regions than the lower regions after bronchodilator inhalation, although the initial and post bronchodilator values did not differ statistically ($p>.05$). The ratio of RVr/TLCr in the upper lung regions (5 cm below the top of the lung) to that in the lowest (29 cm) regions (U/L ratio) was 1.60 ± .50 initially, 1.50 ± .49 post-methacholine and 1.90 ± .53 (mean ± 1SD) post bronchodilator. The U/L ratio post - bronchodilator differed significantly from the initial ($p<.05$) and post-methacholine ($p<.001$) values. The numerical difference between the highest and lower lung regions studied increased slightly from .13 ± .09 initially to .16 ± .14 post-methacholine and .19 ± .11 (mean ± 1SD) post bronchodilator, although this did not represent a statistically significant change ($p>.05$).

Six subjects (4 patients and 2 volunteers) showed a 25% or greater decrease in $\dot{V}_{50}$, $\dot{V}_{75}$, $\dot{V}_{50}$isovol or $\dot{V}_{75}$isovol without a significant fall in FEV_1. This was associated with a 18-20% increase in RVr/TLCr in all lung regions, although achieving statistical significance ($p<.05$) only in the region 11 cm below the top of the lung. The post bronchodilator results differed significantly ($p<.05$) from the post-methacholine values only in the lowest (29 cm) lung

region studied. The U/L ratios were 1.72 ± .39 initially, 1.60 ± .41 post-methacholine and 1.75 ± .51 (mean ± 1SD) post bronchodilator in this group. The numerical difference between upper and lower region RVr/TLCr measurements did not alter between the three studies.

Eight subjects (1 patient and 7 volunteers) had no change in FEV_1 after methacholine, although a >25% decrease in $\dot{V}_{75isovol}$ was observed in 5 of these individuals. The RVr/TLCr measurements did not alter after methacholine inhalation or following bronchodilator in this group. The U/L ratios were 1.79 ± .39 initially, 1.65 ± .26 post-methacholine and 1.72 ± .34 post bronchodilator. The numerical difference between the upper and lower region RVr/TLCr measurements remained constant between the three studies.

A patchy change in RVr/TLCr was observed post challenge in the methacholine responsive subjects, differing in severity between the two lungs. This could either be predominantly an upper zone change or predominantly a lower zone change or a generalized increase. In some subjects, one (or more) lung zones showed no change in RVr/TLCr post-methacholine despite significant changes in adjacent lung regions. This observation is not apparent from the mean results for the subjects which reflect the average changes from several subjects with upper, several with lower and some with generalized changes in RVr/TLCr. These patchy changes are similar to those observed previously in

asthmatics and demonstrate that methacholine inhalation may affect any airways, regardless of their specific anatomical locations, in either a localised or generalized manner.

These findings support the concept that methacholine alters the critical airway opening and closing pressures in a patchy manner, and that there is no single critical opening or closing pressure for all alveoli in the lungs. This change may be reversed by bronchodilator inhalation. The observation that post bronchodilator U/L ratios were not significantly greater than initial U/L measurements in any subject group differs from the findings of Engel et al (J. Appl. Physiol. 1976; 40:411). The differences between the results of Engel et al and these results, particularly in the changes in U/L ratios, may be due to the different methods of aerosol delivery used leading to different deposition patterns. The effect of increased bronchomotor tone on regional lung function is clearly demonstrated by this study, as is the value of inhalational challenge testing to assess the level of nonspecific airways reactivity.

ACKNOWLEDGEMENTS

I gratefully acknowledge the help I have received from my Supervisors, Drs. Richard Jones and Brian Sproule, throughout the course of this project. My thanks also to my advisors, Drs. Tom Overton and Paul Man.

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GLOSSARY OF TERMS AND ABBREVIATIONS

AMMD	- Aerodynamic mass median diameter, μm. The diameter of a unit density sphere that has the same settling velocity as the particle in question.
AMP,cyclic	- Cyclic adenosine monophosphate
BD	- Bronchodilator
BHT	- Breath hold time, s
Bq	- Becquerel; 3.7×10^{10} Bq = 1Ci = 37 GBq
CC	- Closing capacity, %TLC
Ci	- Curie ($1Ci = 3.7 \times 10^{10}$ Bq)
cm	- centimeter
CO	- Carbon monoxide
Cot	- Cotangent
cps	- Scintillation counts per second
CV	- Closing volume, %VC
D_LCO	- Diffusion capacity of carbon monoxide across the alveolar - capillary membrane, ml/min/mmHg.
Dose units	- 1 dose unit is 1 breath of 1 mg/ml methacholine solution inhaled from a standardised aerosol delivery system.
ERV	- Expiratory reserve volume, ℓ
ERVr	- Regional expiratory reserve volume, %TLCr
ET-FEV_1	- End tidal forced expiratory volume in 1 second, %ERV (Normal>75% ERV)

F_ACO	- Fractional concentration of carbon monoxide in alveolar air
F_AHe	- Fractional concentration of helium in alveolar air
FEF_{25-75}	- Forced expiratory flow measured between points 25 and 75% of forced vital capacity, ℓ/s
FEV_1	- Forced expiratory volume in 1 second, ℓ or %VC (Normal>75% VC)
F_ICO	- Fractional concentration of carbon monoxide in inspired gas
FIF	- Forced inspiratory flow, ℓ/s
F_IHe	- Fractional concentration of helium in inspired gas
FRC	- Functional residual capacity, ℓ
FRCr	- Regional functional residual capacity, %TLCr
GBq	- Giga Becquerel; 10^9 X 1Bq
GMP,cyclic	- Cyclic guanosine monophosphate
He	- Helium
^{133}I	- Iodine-133
IC	- Inspiratory capacity, ℓ
ICr	- Regional inspiratory capacity, %TLC
KeV	- Kilo-electron volts
kg	- kilogram
kVp	- kilovolt peak
ℓ	- liters
MBq	- Mega Becquerel; 3.7 X 10^{10} Bq = 1Ci

mCi	- MilliCurie; 1mCi = 37 MBq
MEFR	- Maximum expiratory flow rate, ℓ/s (Also termed PEFR)
Methacholine	- Acetyl-beta-methyl choline
mg	- milligrams
MIFR	- Maximum inspiratory flow rate, ℓ/s (Also termed PIFR)
ml	- milliliter
mRad	- Milli-Rad; Rad = Radiation absorbed dose. 1 RAD is a unit of measurement of absorbed dose of ionising radiation.
NaCl	- Sodium chloride
$NaHCO_3$	- Sodium bicarbonate
N_2	- Nitrogen
O_2	- Oxygen
p	- probability of obtaining the observed or greater variation by chance alone.
P_{Aco}	- Alveolar partial pressure of carbon monoxide
P_B	- Barometric pressure, mmHg
ΔP	- Change in pressure P, mmHg
$P_{\bar{C}co}$	- Partial pressure of carbon monoxide in pulmonary capillary blood
PC_{20FEV_1}	- The concentration of inhaled methacholine solution (mg/ml) at which a 20% decrease from the control FEV_1 value is observed.

PD_{20FEV_1}	- The cumulative dose units administered up to the point at which a 20% fall in FEV_1 from the control value is observed.
PEFR	- Peak expiratory flow rate, ℓ/s (Also termed MEFR)
PIFR	- Peak inspiratory flow rate, ℓ/s (Also termed MIFR)
psi	- Pounds per square inch pressure
r	- Correlation coefficient
Raw	- Airways resistance $cmH_2O/ℓ/s$
Reactivity	- The airways reactivity to an inhaled agent is the rate of decrease of the observed parameter (eg. FEV_1) with increasing dose administered (i.e. the slope of the dose response curve).
rho	- σ, population standard deviation
RV	- Residual volume, ℓ
RVr	- Regional residual volume, %TLCr
s	- second
SD	- Standard deviation
SEE	- Standard error of the estimate
SEM	- Standard error of the mean (SD/$\sqrt{n}$,where n is the number of observations).

Sensitivity - The airways sensitivity to an inhaled agent is expressed as the concentration (or cumulative dose) required to produce a predetermined decrease in an observed parameter from its control value (eg. $PC_{20FEV_1}/PD_{20FEV_1}$).

Tan - Tangent

TLC - Total lung capacity, ℓ

TLCr - Regional total lung capacity (usually expressed as cps at TLC for given lung region)

U/L - Ratio of RVr/TLCr in the upper lung zones (1 and 6) to RVr/TLCr of the lower lung zones (5 and 10).

ΔV - Change in volume V, ℓ

$\dot{V}$ - Flow rate, ℓ/s

$\dot{V}_{50}$ - Flow rate measured 50% below TLC during a maximum forced expiration from TLC, ℓ/s

$\dot{V}_{75}$ - Flow rate measured 75% below TLC during a maximum forced expiration from TLC, ℓ/s

$\dot{V}_{50isovol}$ - Isovolume $\dot{V}_{50}$, ℓ/s or % of control $\dot{V}_{50}$

$\dot{V}_{75isovol}$ - Isovolume $\dot{V}_{75}$, ℓ/s or % of control $\dot{V}_{75}$

V_T	- Tidal volume, ℓ
$\dot{V}_{10}/V$	- Ventilation per unit volume at 10 breaths/minute
$\dot{V}_{60}/V$	- Ventilation per unit volume at 60 breaths/minute
$\dot{V}w/V$	- Washin ventilation per unit volume
$\bar{x}$	- mean of x
^{133}Xe	- Xenon-133

Plate 1: The Walter C. Mackenzie Health Sciences Centre, University of Alberta Hospital, Edmonton. (Photograph by Joe Twyman).

Chapter I

THE PROBLEM

(i) Background to the problem

A recent longitudinal survey by Dodge and Burrows (1) demonstrated that, from a general population sample of 3,860 subjects (791 under 15 years of age), 38% had at some time experienced an attack of shortness of breath with wheeze. Of the total population sample, 6.3% had documented asthma and 2.7% chronic bronchitis alone (2). Some of the asthmatics also had symptoms of chronic bronchitis. A potential source of bias in this study was that the climate in Arizona attracts subjects with lung disease, particularly in the older age group, around 75% of asthmatics having had their disease before moving to Arizona. Unfortunately there are few other thorough studies of incidence and prevalence of asthma, the lack of uniformity of diagnostic criteria having restricted research in this area.

Another survey of 385 consecutive asthmatic patients (defined in terms of reversible airflow obstruction) showed that 98 (25%) also fulfilled the MRC criteria for chronic bronchitis (3,4). Further data is needed using the now generally accepted criteria of reversibility of airflow obstruction to define the incidence and prevalence of asthma in other countries. These two reports emphasise the overlap between asthmatic and bronchitic symptoms and also the frequency of wheezing in a large population sample - even after excluding those with respiratory disease prior to moving to the area in the Arizona survey.

A carefully taken history and physical examination,

sometimes supplemented by a few well chosen investigations, will generally reveal the basis for the patient's complaint, particularly if there is a smoking history. A chest radiograph may suggest emphysema, this representing the "destructive" end of the chronic lung disease spectrum. Blood eosinophilia accompanying wheeze in an atopic individual makes asthma the probable diagnosis, although pulmonary function testing should still be performed to confirm bronchodilator responsiveness of airflow obstruction.

For the majority of patients presenting with respiratory symptoms achieving a diagnosis presents little difficulty. There remain, however, a significant number of individuals with intermittent respiratory symptoms who cannot be confidently categorised with the routine approach mentioned above. The identification of those with episodic asthma is of great importance in this patient group, both in terms of offering appropriate therapy and in attempting to prevent subsequent unnecessary pulmonary deterioration if left untreated.

(ii) Statement of the problem

When the physical examination findings and laboratory investigations confirm the diagnosis suggested by the patient's story, the management is relatively clearcut. Supplementary investigations may be helpful for those individuals presenting with a history of recurrent episodic

wheeze, cough or breathlessness but a negative physical examination. The existence of "asthma without wheeze" has been recognised for some time (4,5,6) and offers a special diagnostic challenge.

Bronchial inhalational challenge testing is of particular value in assessing these episodic symptoms suggestive of asthma. The basic intention of challenge testing is to see if the subject's symptoms can be reproduced under supervision in the laboratory setting and to observe the effects on lung function. A specific antigen may be chosen if there is a probable occupational or environmental factor. Alternatively, airways sensitivity may be assessed by inhalational challenge testing with nonspecific agents such as methacholine chloride. Since we know asthmatics are 100 to 1000 times more sensitive to methacholine than normals (7), the demonstration of airways hypersensitivity in a patient with normal physical and pulmonary function test data can be extremely helpful in clarifying the diagnosis.

Relatively little work has been done to establish how inhaled broncho-provocation agents affect regional lung function (8,9,10). Since patients with episodic bronchospasm usually have normal lung function when examined, their underlying reginal lung function may also be predicted to be normal between episodes of bronchospasm. Asthmatics, however, are known to have altered regional lung function even when in remission, the progression of these

changes with time correlating with changes in overall lung function (11,12). Patchy change in regional lung function following methacholine challenge, similar to those present in asthmatics, would further support the diagnosis of "episodic asthma" in those patients with intermittent respiratory symptoms.

(iii) Research objectives and hypothesis

The intention of this study was to assess the response to inhaled methacholine in a group of patients with symptoms suggestive of episodic bronchospasm but normal physical examination and pulmonary function data. To gain further information about the site and basic mechanisms of action of methacholine, as well as to increase the sensitivity of the study, regional RV/TLC ratios (RVr/TLCr) were also assessed using Xenon-133 radiospirometry on a multi-detector system. Five zones for each lung were assessed on 3 occasions: (1) immediately prior to challenge testing, (2) immediately following challenge testing and (3) following bronchodilator administration.

Stating the null hypothesis to be tested:

"There is no difference in response to nonspecific bronchial provocation with inhaled methacholine chloride between subjects with normal baseline lung function data but a history suggestive of episodic airflow obstruction and normal healthy volunteers without preceding respiratory symptoms".

The hypothesis was therefore tested in two ways. Firstly, by assessing airways sensitivity to methacholine in the group with symptoms as compared to healthy volunteers. Secondly, by comparing the changes in regional lung function data obtained from the two groups.

Chapter II

A REVIEW OF THE LITERATURE

(i) Bronchial Inhalational Challenge Testing

a) A historical perspective

The pathognomonic features of bronchial asthma are markedly increased airways hypersensitivity and reversible airflow obstruction. In remission the degree of airflow obstruction may be minimal, or pulmonary function tests entirely normal. Airways hyperreactivity to inhaled bronchoconstrictor agents - whether occurring naturally in the environment or administered as challenge tests in the laboratory setting - remains elevated even whilst the asthmatic is in remission, the degree of responsiveness correlating closely with the presence and severity of asthma (13,14). The true "challenge" in bronchial inhalational challenge testing is therefore in determining increased airways reactivity in the absence of the features of asthma.

Increased awareness of the deficiencies in knowledge relating to the pathogenesis of bronchospasm has stimulated much research using bronchial provocation tests. This has also been facilitated by advances in scintigraphic techniques for determining the precise sites of aerosol deposition, as well as more sophisticated methods for assessing their effects.

Despite the proliferation of literature on challenge testing over the last decade, this is not a new technique. In 1912 the opposing effects of subcutaneous pilocarpine and atropine in subjects with bronchial asthma were reported by Barker and Sladen (15). Alexander and Paddock reported a

study on 20 clinically well asthmatic patients in 1921, demonstrating that an "asthmatic-like" attack could be induced in half of these subjects by a subcutaneous injection of pilocarpine (16). Inadvertently Starr et al in 1933 precipitated an "asthmatic-like" attack in a healthy young subject who had a previous history of asthma (although free from symptoms for several years) following subcutaneously administered acetyl-beta-methyl choline (17). Starr subsequently showed that methacholine taken orally by asthmatics would also precipitate mild bronchospasm as well as various other non-respiratory effects (18,19).

In 1940 Moll reviewed the literature regarding the action of parasympathomimetic drugs in asthma (20). He concluded that the peculiar sensitivity of asthmatics to the choline derivatives was due to enhanced local sensitivity in the lungs, not just part of a generalised sensitivity reaction. This was supported by the observation that the systemic effects of the cholines were no different in asthmatics from normals. Moll considered that preceding pathologic lung damage in asthmatic subjects was an essential factor in determining the abnormal bronchial response.

Asthmatics have therefore been known to be sensitive to inhaled, oral and parenterally administered agents for at least 70 years, but the first major reports on the use of inhalational agents in the diagnosis of asthma were in the

1940's. Dautreband and Philippot (21) demonstrated that "asthmatic-like attacks" could be induced with inhaled aerosols of carbaminocholine and relieved with an aerosol of phenyl-amino-propane. Thus asthma was clearly shown to be precipitated by inhalational agents, with the wheezing and airflow obstruction being reversible.

Substernal discomfort and coughing was reported in normal subjects following the administration of intravenous or intramuscular methacholine (22,23) associated with a fall in vital capacity (24). Curry showed that hay fever sufferers had increased sensitivity to methacholine as compared to normals, although this was less dramatic than in asthmatics (25). Nebulised methacholine at 1:40,000 concentration was found in this study to induce a more severe fall in vital capacity than when larger amounts were administered intramuscularly or intravenously. Curry concluded that nebulisation was the most appropriate method for assessing airways sensitivity, the lungs being particularly sensitive to this route of administration.

Many of the early aerosol studies used rather cumbersome apparatus, so the modification introduced by Parker et al in the 1960's (26) made aerosol challenge testing a much more practical diagnostic test for asthma. Inhalational challenge testing has now been widely accepted as a useful test for asthma, as well as providing a mechanism for studying airways function in general (27). The subsequent review considers some of the factors which

influence the response to a challenge test, as well as considering the situations where bronchial provocation testing is appropriate.

b) Indications for inhalational challenge testing

Tests of bronchial reactivity may be considered either for diagnostic or research purposes. The diagnostic role is quite narrow, challenge testing being appropriate only where there is a suspicion of asthma in a spirometrically normal individual, or where documentation of an environmental precipitating factor is essential for subsequent patient management (28,29).

Since we know that asthmatics show markedly elevated nonspecific airways responsiveness, challenge testing is considered by many to be inappropriate in subjects with clear evidence to support the diagnosis of asthma (30). Woolcock (31) contends, however, that if we do not estimate airways reactivity in our asthmatic subjects, then our understanding of the mechanisms involved in nonspecific responsivenss will not progress. She states in a deliberately provocative comment that the measurement of nonspecific airways reactivity

> "should be as essential to the diagnosis and management of asthma as the glucose tolerance test is to diabetes" (31).

Juniper et al (32) also suggest that assessment of airway responsiveness may be appropriate in established

asthmatics to monitor progress with therapy. The level of responsiveness appears to correlate well with medication requirements. Defining the PC_{20FEV_1} as the concentration of methacholine which produced a 20% fall in FEV_1 (a positive challenge test), they observed that a PC_{20FEV_1} of greater than 2 mg/ml methacholine in their asthmatics was generally associated with minimal respiratory symptomatology. Such patients were able to reduce to a less intensive medication regime, using prn bronchodilators with or without prophylactic Beclomethasone, without deterioration in their asthmatic control. Conversely, when the PC_{20FEV_1} was less than 2 mg/ml methacholine and the patient on no, or minimal, therapy, they concluded that medications should be instituted or a trial of more intensive therapy considered.

There are several other areas of research applicability. The long-term stability of nonspecific airways responsiveness, in the absence of intercurrent exacerbating factors (14), provides a useful model for evaluating the effect of drugs on the airways. Salbutamol therapy, for example, will acutely decrease airways responsiveness, as will adequate therapeutic doses of oral theophyllines (33). New medications may therefore be compared using nonspecific airway reactivity as an index of their success. Factors affecting bronchial responsiveness are considered later in this chapter (p.22).

c) Methods of aerosol delivery

Several different techniques are employed by different laboratories, making direct comparison of results in the literature difficult. They involve either intermittent or continuous aerosol generation techniques.

Continuous aerosols are generally administered via a facemask during tidal breathing. The dose of aerosol delivered is calculated as the product of respiratory frequency, tidal volume, duration of nebulisation and nebuliser output. This is a relatively wasteful technique, aerosol being generated all the time but being inhaled only part of the time (i.e. dependent on the respiratory rate).

The most useful continuous aerosol method uses a reservoir bag to collect the aerosol mist, the subject inhaling from the bag via a one-way valve, exhaling through a separate expiratory valve. This method conserves aerosol (particularly important in aerosol deposition studies using radioactive isotopes) and also allows deposition of larger aerosol particles in the reservoir bag, the subject inhaling only the smaller particles. The significance of particle size on aerosol deposition is discussed below.

Methods of intermittent aerosol generation require greater patient cooperation. A dosimeter is used to regulate the frequency and volume of aerosol delivery. It is important to regulate aerosol delivery to occur in the latter part of inspiration, since the higher flow rates of early inspiration lead to predominantly upper airway

particle deposition (34,35). A relatively constant and reproducible level of aerosol deposition throughout the bronchial tree can then be achieved from inhalation to inhalation.

d) Factors influencing the deposition of inhaled particles

Aerosol deposition in the respiratory tract depends on the particle size, inspiratory volume, inspiratory flow rate and the subsequent breathholding pause. Variation of any of these factors will alter the amount of aerosol reaching each lung region. It is therefore vitally important to standardise the aerosol delivery technique for any clinical study on inhalational challenge testing.

Particle Size

Most aerosolised drugs have particle sizes in the range 1-5 μm (aerodynamic mass median diameter, AMMD) (36). In this range diffusion, sedimentation and impaction are the most important mechanisms of deposition within the respiratory tract (37,38). Large particles in the 5-10 μm AMMD range are predominantly deposited by inertial impaction in the major airways. During mouth breathing this is predominantly in the tonsilar areas of the pharynx and larynx, but also in the initial five or six bronchial bifurcations (39). This happens because the relatively high inertial forces of large particles limit their ability to follow the airstream as it changes direction.

Gravitational sedimentation accounts for most of the

deposition of 1-5 μm AMMD particles. Around 15-20% of 3 μm particles are nevertheless deposited by impaction in the major airways (40,41). Maximal deposition of these particles, representing the size produced by most nebulisers and pressurised cannister inhalers, is at the 15th to 17th bronchial divisions (40,41). In one study using radioactively labelled 3 μm particles, 15% of particles were deposited in the proximal airways and removed rapidly. The remaining 80% was removed with a much slower clearance rate, 50% still being present after 24 hours (41). This indicates deposition on the peripheral nonciliated airways distal to the terminal bronchiole may be achieved with the majority of aerosol inhaled if the particles are small.

Very small particles, less than 1 μm AMMD, are carried by Brownian diffusion, a random migratory movement of particles in the airstream. These particles remain airborne with little tendency for deposition in the lungs and do not constitute a significant useful component in therapeutic aerosols (39,42).

The presence of airways obstruction has a major effect on the penetration of particles into the lungs, markedly reducing peripheral deposition (39). Deposition data for normal subjects in relation to different particle sizes is illustrated in Figure 1. The particles produced by the DeVilbiss Number 45 nebuliser used in the study reported in this thesis have been estimated to be in the range 0.3-2.0 μm diameter (DeVilbiss Inc., Personal Communication).

This is also in accordance with the data from Logus (42). Figure 2 shows the relative penetration of different sized inhaled particles into the lungs in terms of the bronchial generation number.

The above data relates to work undertaken with nebulisers such as the DeVilbiss number 40 and 45. Pressurised cannister aerosols used for the delivery of medication appear rather less efficient. Newman et al (45) estimated that 80% of the "metered dose"is deposited in the mouth, only 8.8% on average reaching the lungs (3.0% to the alveoli and 5.8% in the conducting airways). Of the remaining dose, 1% was exhaled and 9.8% deposited in the aerosol actuator.

Mode of inhalation

Dolovich et al demonstrated maximal penetration of 0.55 μm AMMD particles when inhaled with inspiratory capacity breaths each followed by a 10 second breathhold (45). Aerosol penetration was seen to be reduced in the presence of airways obstruction, this being of relevance in the interpretation of the site of action with inhaled methacholine and other bronchoconstrictive agents (46,47).

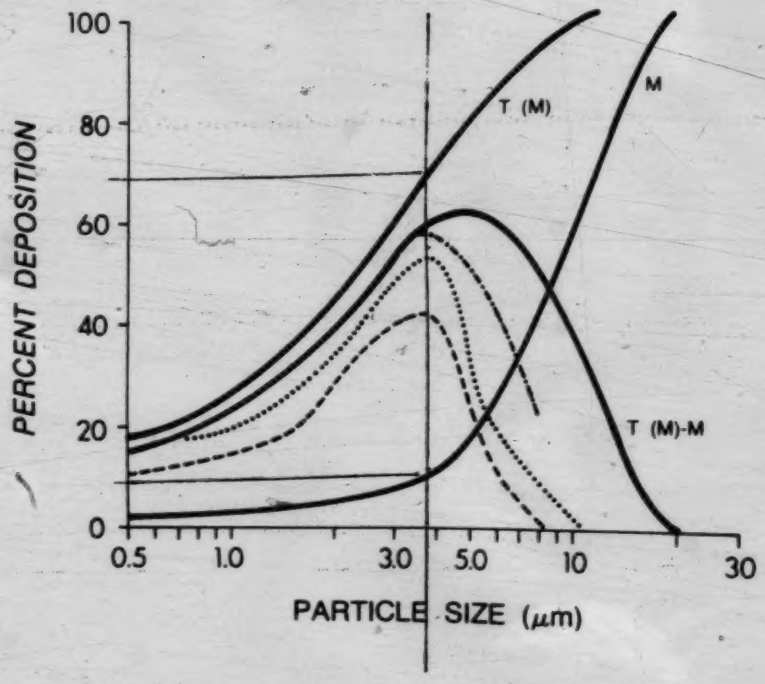


Fig. 1 - Particle deposition in the lungs in relation to particle size for mouth breathing.

The average curves for total particulate deposition in the respiratory system, T(M), mouth deposition, M and tracheo-bronchial deposition including alveolar, T(M) - M, are shown. Three different estimates of alveolar deposition (broken lines) are depicted, one (-•-•-) suggesting zero tracheo-bronchial deposition for particles less than 3 μm. Tidal volume was 1000 ml and respiratory rate 10 breaths per minute.

Data from Morrow (43) with permission from Chest.

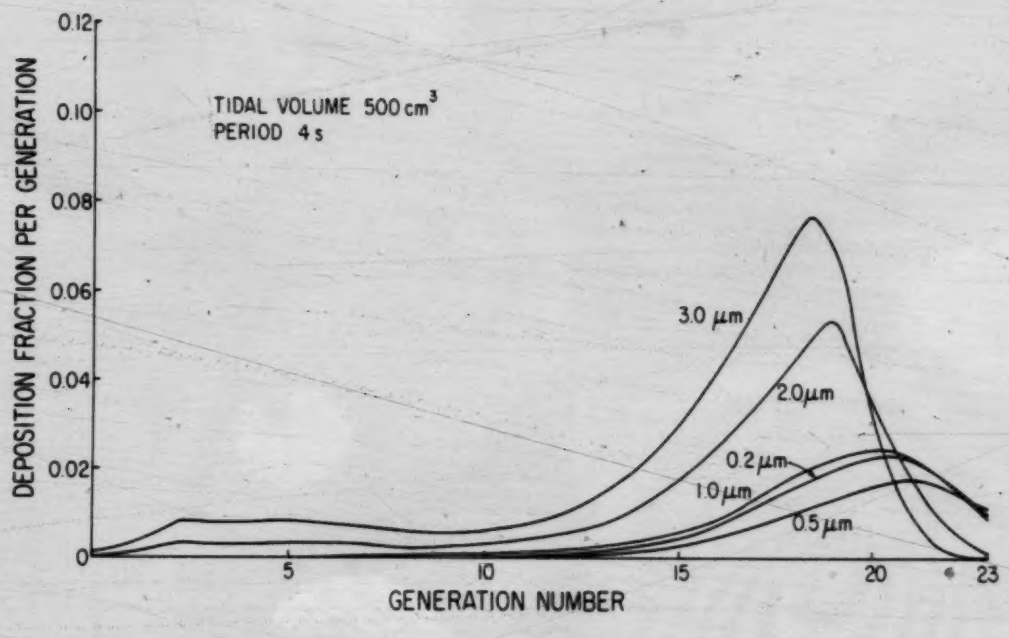


Fig. 2 - Theoretical prediction of deposition profile along the respiratory tract at 500 ml tidal volume and 4 second breathing period for various particle diameters.
Reprinted with permission from Perganon Press and the Institute of Occupational Medicine (44).

Generation 0 = trachea
1-4 = main bronchus to subsegmental bronchi
5-15 = small bronchi to terminal bronchioles
16-23 = respiratory bronchioles, alveolar ducts and alveolar sacs
24 = alveoli

The inertia of inhaled particles is increased at high inspiratory flow rates, effectively increasing the aerodynamic particle size, resulting in increased proximal airways deposition (39). Optimal peripheral deposition with intermittent aerosol generation techniques is obtained when the aerosol is delivered in the latter half of inspiration (34,35). It is therefore possible to regulate the site of aerosol deposition by varying the particle size, inspiratory flow rate and timing of aerosol delivery during the inspiratory cycle. Thus an understanding of the physical principles underlying aerosol delivery has facilitated studies of the site of action of inhalational agents, as well as provided opportunity for study of specific deposition characteristics in disease states (38,39).

e) Inhalational agents and their effects

The relative advantages of specific antigenic challenge over nonspecific challenge with methacholine or histamine have been summarised by Spector and Farr (48). Methacholine and histamine challenge serves only to document the level of general airways irritability (reactivity) without giving clear indication as to the etiology. For the diagnosis of asthma this is adequate in view of the marked sensitivity of asthmatics to nonspecific inhalational challenge (7).

When an environmental factor is suspected, challenge with specific antigens can help to establish a direct causal relationship. This is particularly important in

occupational asthma, where definite identification of the causative factor influences advice about career choice or a change of occupation. In seasonal asthma specific antigenic challenge can also be helpful, especially if desensitisation is contemplated.

Airways reactivity to antigenic and nonspecific agents are closely correlated, asthma being produced more readily by inhaled allergens if nonspecific bronchial reactivity is markedly elevated (49). The ability of medication to block or reduce airways reactivity may therefore be assessed by either allergenic or nonspecific challenge.

Bronchial hyperreactivity to inhalation of ultrasonically nebulised solutions of distilled water or saline has also been demonstrated. Significant fall in FEV_1 resulted from inhalation of hypotonic and hypertonic, but not isotonic, saline solutions in ten asthmatics (50). This emphasizes the need for including inhalation of the diluent as a control in all challenge tests.

Response to antigenic challenge is either an immediate (Arthus type I) or delayed (Arthus type III) hypersensitivity reaction (51). Airways resistance and residual volume increase; forced vital capacity and forced expiratory flow rates fall. If the response is severe, dyspnea, wheezing and chest tightness may be experienced. The immediate type reactivity to methacholine or histamine produced similar effects to antigenic challenge, but no delayed reaction has been reported (7). The effect of

nonspecific challenge generally wears off within 30-45 minutes without bronchodilator, or reverses to normal within 5-10 minutes after bronchodilator (7).

The minimum changes necessary to accept a challenge test result as significant vary according to the test used. Generally a 20% fall in FEV_1 is accepted as significant, but other criteria include a 10% fall in FVC, a 25% fall in $\dot{V}_{50}$, $\dot{V}_{75}$ or maximal mid-expiratory flow rate (FEF 25-75%), a 40% fall in specific airway conductance and a 25% increase in FRC (35). The magnitude of the change in specific conductance required for significance is due to the marked influence of suggestion on body plethysmograph measurements. Unfortunately those individuals found to respond most to bronchoconstrictive suggestion are also those with most highly reactive airways (52). The FEV_1 response was not shown to be affected significantly by suggestion. Inhalation to TLC prior to performing a forced expiration may, however, reverse bronchospasm in normals and accentuate it in asthmatics (53,54).

Methacholine is a parasympathomimetic drug which stimulates acetyl choline receptors on bronchial smooth muscle cells to produce bronchoconstriction when inhaled in an adequate dose. Inactivation of methacholine is by cholinesterase. Parenteral or inhaled atropine and inhaled bronchodilators can prevent or reduce the response to methacholine, a finding not consistently observed with histamine-induced nonspecific airways reactivity (7,35).

f) Airways responsiveness in health and disease

Asthmatics with the highest airways responsiveness to nonspecific inhalational challenge are hospitalised most frequently, particularly those who attempt to disregard or deny their breathing difficulties (55). The sensitivity to methacholine of asthmatics has been estimated as 100 to 1000 times that of normals, thus making the degree of sensitivity useful in both defining asthma and as a genetic marker for the disease (7).

Many factors can modify the level of airways reactivity. Suggestion may either increase or decrease the level of responsiveness (52), being a particularly important factor in determining the percentage fall required for significance in the various parameters of lung function measured (see preceding section). Recent viral infections of the respiratory tract enhance nonspecific reactivity, an effect which can be blocked by prior atropine aerosol administration (56,57). Vaccination with live attenuated measles virus produces increased airways sensitivity to methacholine, reaching a peak level of reactivity 4 weeks after vaccination before slowly returning to normal over the next 1-2 months (58). In a measles epidemic, however, decreased airways reactivity was demonstrated from pre-infection levels (58). A possible explanation would be that the nature of the virus in the attenuated measles vaccine was altered. The result is still surprising, however, since the more virulent natural virus might have been expected to

produce greater change than an attenuated virus.

Inhaled bronchodilators acutely reduce airways reactivity (7,25,31,32), thus these should be withheld for an adequate period prior to challenge (35). Adequate therapeutic doses of oral theophyllines also reduce nonspecific airways reactivity, although subtherapeutic doses (blood level below 10 mg/ℓ) were found not to offer a protective effect against histamine challenge (33). Oral corticosteroids do not consistently affect the threshold of methacholine reactivity, 27 out of 37 individuals having the same or increased nonspecific airways reactivity as compared to presteroid therapy levels (59). These various factors affecting airways reactivity are summarised in Table 1 (p.49) and recommended medication-free intervals prior to challenge testing in Table 2 (p.50).

Subjects with hay fever are found to have slightly increased airways reactivity, although not of the magnitude observed in asthmatics. Less than 5% of individuals with hay fever or who are nonatopic normals show high levels of airways reactivity. In contrast, over 90% of asthmatics have markedly increased sensitivity to nonspecific challenge (7). These observations are interesting, as specific allergenic challenge in patients with ragweed allergic hay fever and ragweed allergic asthma cannot distinguish between these two categories, whereas methacholine challenge can do so quite effectively (34). In a study of over 1500 asthmatics, Townley et al recorded no negative methacholine

challenge tests, although the level of reactivity varied between individuals (7).

Increased airways reactivity is also reported in other diseases. Bronchoprovocation has been recommended for the diagnosis of hypersensitivity pneumonitis due to a variety of bacterial, fungal, protein and chemical antigens (51,59). In cystic fibrosis increased nonspecific airways reactivity has been reported in 24% of patients, although positive responders to challenge were not evenly distributed over the entire disease spectrum (61). The positive responses occurred only in those subjects with abnormal lung function pre-challenge, the heightened bronchial reactivity reflecting the severity of their underlying lung disease rather than the presence of co-existent asthma. Van Asperen et al (62), however, studied a group of 20 children with minimal changes in pulmonary function tests; eight (40%) challenge tests were positive, 4 of these being comparable to the level of reactivity observed in asthmatics. In the presence of minimal derangement of lung function, they considered that a strongly positive nonspecific challenge test did provide evidence of co-existent asthma with cystic fibrosis.

g) <u>The site and pathogenesis of bronchial reactivity</u>

Many studies have shown that the extra-pulmonary responses to methacholine and histamine (e.g. flushing, sweating, tachycardia, salivation) are similar in asthmatics

and nonasthmatics (17,19,29,25). Moll therefore deduced that the marked change in lung function observed in asthmatics following administration of these agents was not part of a generalised (organ non-specific) susceptibility (20). He suggested that pre-existent lung damage in asthmatic subjects led to loss of an airways protective mechanism, then undefined, resulting in abnormal bronchial responsiveness. Subsequent observation that normal subjects may also experience a feeling of substernal constriction, coughing and a fall in VC showed that, whilst lung damage might be present, it was not the sole reason for increased bronchial responsiveness in asthmatics (24,25).

Eppinger and Hess in their treatise on "Vagotonia" suggested that asthma was an example of pathologically increased vagal tone (63). Curry, however, found that whilst intravenous atropine sulfate would give some relief in asthmatics, parenteral epinephrine was far more effective (25). He concluded that increased vagal tone was not a major factor in determining airways reactivity. If he had used inhaled atropine sulfate his conclusions may well have been different, since we now recognise that this can be useful therapy in some, although not all, asthmatics (56).

Empey et al (56) suggest that the primary abnormality in bronchial hyperreactivity is on the afferent side of a reflex arc mediated through the vagus nerve. Perhaps this could explain why increased airways reactivity has been observed in close non-asthmatic relatives of asthmatics,

particularly if they are themselves atopic (64). Fallier et al, however, found that in monozygotic twins one member may have grossly hyperreactive airways and asthma whilst the other twin is asymptomatic and does not have enhanced nonspecific airways reactivity (65). Thus asthmatics are not necessarily born with hyperreactive airways, but might inherit a predisposition towards developing hyperreactivity, this being triggered in response to an environmental stimulus such as a viral or bacterial respiratory infection (56,57,59). This would explain the clinical situation where pneumonia may result in the subsequent development of asthma in a non-atopic individual.

Activation of receptors in the upper airway, such as by cold air stimulation as implicated in exercise induced asthma, causes coughing and bronchoconstriction (6,66). Vagal stimulation with subsequent acetyl choline release stimulating bronchial smooth muscle constriction may well play a major role in this sequence, coughing producing increased output from airways irritant receptors leading to a positive feedback effect. Reflex bronchoconstriction followed by more coughing and increasing airways obstruction results. Inhaled atropine sulfate has been shown to either prevent or reverse this response (56). The significant effect of suggestion on airways conductance (SGaw) also supports a role for a vagal pathway. Spector and Kinsman (52) suggest that information (in this case suggestion) is relayed from the cerebrum to the hypothalamus and thence via

the vagus to the tracheo-bronchial tree.

Atropine is most effective in inhibiting methacholine-induced bronchospasm, with little effect on histamine inhalational challenge (7). Thus histamine sensitivity, which generally correlates closely with methacholine responsiveness, does not appear to be vagally mediated. Townley et al suggest instead that these inhalational agents produce bronchoconstriction because the beta-adrenergic receptors, which normally maintain broncho-dilation, are deficient or defective (7).

Modulation of airways smooth muscle reactivity may therefore be considered to be through the combined effects of beta-2, alpha and cholinergic receptors regulating the levels of cyclic-AMP and cyclic-GMP (50,67-72). Mast cell-derived mediators of immediate (type I) hypersensitivity have also been identified in the airways and may produce direct and reflex effects on the epithelium and bronchial smooth muscle (68). The role of the mast cell and the prostaglandin and kallikrein systems in the pathogenesis of airways reactivity clearly warrants further investigation. The current state of knowledge regarding the interrelationship of these factors is well reviewed by Kaliner (68).

The site of airways response in atopic non-asthmatics appears to be different from that in asthmatics. Fish et al (9) found that hypersensitivity in asthmatics was in both central and peripheral airways, whereas in subjects with hay

fever (but not asthma) the large central airways were predominantly affected. This conclusion was based on the finding that when a similar degree of fall in SGaw was produced in the two groups of subjects, no corresponding change in FEV_1 was present in the atopic non-asthmatics. Cholinergic smooth muscle receptors were shown in cats to influence bronchomotor tone in the large and medium sized airways, as well as affecting the mechanical properties of the respiratory bronchiole and alveolar duct (73). Fish and co-workers (9) therefore suggested that the difference in site of response between the asthmatics and hay fever subjects might be due to differences in cholinergic reactivity between the two groups at different levels in the airways.

Newhouse and Ruffin (39) demonstrated that by adjusting the aerosol particle size and mode of delivery to give mainly central airways deposition, much less inhaled histamine was required to produce a 20% fall in FEV_1 than when the delivery technique favoured a more diffuse intrapulmonary deposition pattern. The FEV_1, however, is not a sensitive way of assessing the effect of inhalational challenge on peripheral airways. The observations may therefore either be due to the histamine-sensitive receptors being predominantly in the larger central airways, or just that the peripheral receptors did not receive a large enough dose to be affected by the diffuse aerosol deposition technique. Administration of salbutamol prior to histamine

challenge provided a similar degree of protection whether a diffuse peripheral or central deposition technique was used for salbutamol administration. If the effects of challenge were monitored by flow-volume loop measurements, diffuse salbutamol deposition was found to have a much greater protective effect on end-expiratory flow rate ($\dot{V}_{75}$) during subsequent histamine challenge than if deposited centrally. Thus the receptors sensitive to non-specific inhalational agents are likely to be diffusely distributed throughout the airways, as in the cat (73), since different levels of protective effect on FEV_1, $\dot{V}_{50}$ and $\dot{V}_{75}$ may be produced with different sites of salbutamol deposition.

It can be seen that, despite the wealth of knowledge relating to airways reactivity, there is still not total agreement on the relative importance of different factors and mechanisms in its pathogenesis. Hopefully further studies with radioactive tracers and specific deposition techniques similar to those of Newhouse and Ruffin (39) will provide a better understanding of this important aspect of airways reactivity.

(ii) The assessment of regional lung function

a) Historical development of techniques

Changes in regional ventilation may be produced by localised lung disease. These may not be detected by routine tests of overall pulmonary function or arterial blood gas analysis until the disease process is relatively advanced. Chest radiographs localise the anatomical site of an abnormality without quantifying the degree of physiologic disturbance produced.

Bronchospirometry, introduced by Björkman in 1934 and further developed by Carlens in 1949 using a double lumen catheter, measures function of the left and right lungs individually, but still does not localise a lesion within a lung (74,75). Modifications of this technique by selective bronchial catheterisation have provided a lot of information on regional lung ventilation (76). These tests are, however, elaborate to perform, uncomfortable for the patient and impose an unphysiological state on the subject - although less than that caused by the larger Carlens' tube. Fowler's single breath nitrogen washout technique provides some information regarding the relative regional inhomogeneity of lung ventilation caused by a disease process (77). Subsequent refinements of this technique have shown it to be useful in detecting early small airways disease (78-82).

The major breakthrough in assessing regional lung function came in 1953 with the development of a technique

using an intravenous injection of radioactive isotope (^{131}I) detected by external scintillation counters (83). Unfortunately the tracer used was so soluble in blood that the background count produced by isotope in the heart and major vessels obscured the washout curve from the lungs. The use of ^{133}Xe overcame this problem and the accurate assessment of regional lung perfusion and ventilation became possible (84).

Xenon-133, an inert gas poorly soluble in blood, has remained the most popular and practical method of assessing regional lung ventilation since its introduction in 1955 (84,85). Knipping (84) showed that regional lung ventilation could be assessed by injecting a bolus of ^{133}Xe dissolved in saline, monitoring its progress through the lungs (i.e. regional lung perfusion) and then watching the washout phase, excretion being entirely by ventilation (due to the poor solubility of ^{133}Xe in blood). Delayed washout is proportional to the degree of regional ventilatory impairment. Krypton-81m is now being suggested for ventilation studies but, in view of its exceptionally short half-life (13 seconds), its use will remain limited to the few institutions with a cyclotron adjacent to the examining suite (86).

A major role of isotope perfusion studies is in the diagnosis of pulmonary thrombo-embolic disease (87). Unfortunately perfusion abnormalities may also be present in association with chronic lung disease, thus a combination of

ventilation and perfusion studies are usually necessary to unequivocally establish the diagnosis of pulmonary embolism if there is coexistent lung disease (87-89). Perfusion studies can also be helpful in the preoperative evaluation of patients for surgical lung resection (90) and other isotope methods are being used to evaluate aspects of metabolic lung function (87).

b) Different detector systems and their limitations

An important factor in choosing a detector system for lung ventilation studies is the attenuation and scatter for low energy radiation as it passes through the lung and chest wall tissues. It has been shown that gamma rays produced by ^{133}Xe (80-KeV) are attenuated by 45% when they pass through 10 cm of normal lung tissue (91). In addition, the varying thickness and tissue composition of the chest wall and possible non-uniformity of the detectors used contribute to further limitations when trying to compare blood flow or lung volumes in different lung regions.

Knipping (83,84) used 16 static Geiger-Müller tubes positioned behind the patient in his initial work. Clearly attenuation of radioactive counts originating from the anterior parts of the lungs was a major limitation of this early system. The technique developed by Friedenberg et al at the University of Alberta was an attempt to overcome this problem (92-94). This system uses anterior and posterior banks of static detectors which the subject either sits or

lies between, the arithmetic mean count from collinear detector pairs being used in subsequent data analysis.

Other workers have used a pair of anterior detectors (one over each lung) which move from top to bottom of the lung during breathholding. This method requires the subject supine, thus the distribution of regional blood flow and lung volumes observed differ from those measured when seated subjects are studied (79,95-97). It suffers from the same problems of attenuation already discussed. Slightly better correlation with anatomical site represented by a given count rate is obtained than with the fixed multiprobe detector systems, since the scanner moves caudally from just above the top of the lungs (zero scintillation counts) over the length of the thorax. Thus, by direct measurement from the point where counts start to increase (i.e. the lung apex), the exact site represented by a given scintillation count rate can be determined. A breathhold of several seconds is necessary for this scanning technique, therefore dyspneic patients cannot be studied if they cannot hold their breath for the duration of the scan. With fixed multiprobe systems measurements may be made during normal respiration.

Neither the fixed nor scanning detector systems provide an image, their results being recorded in terms of count rate versus time and count rate versus distance from the lung apex respectively. This is adequate in research studies but, for patients, most clinicians prefer to see a

hard-copy image relating the count rates to their precise anatomic locations. The advent of the Anger scintillation camera, with its wide field of view and facility for static and dynamic lung images, has made this the instrument of choice in most clinical isotope studies (98,99). A mobile gamma camera, permitting bedside evaluation with isotope techniques, further extended the clinical role of nuclear medicine studies (100). The application of tomography to nuclear medicine will provide even greater information from ventilation and perfusion lung studies when this becomes generally available.

c) The determinants of regional lung volumes

Gravitational forces are important in determining regional lung volumes as well as regional perfusion (79). Studies with ^{133}Xe have shown that the alveolar size at FRC decreases progressively from apex to lung base (101). Also, the lung base is observed to expand more than the apex when a maximal inspiration from FRC is taken. Thus the apical alveoli are relatively hyperexpanded in relation to the compressed basal alveoli, and the ventilation per unit volume is greater in the lower lung zones than the upper zones (102).

In a subject equilibrated on a mixture of ^{133}Xe in air in a closed-circuit spirometer, the concentration (represented by count rates) for a given lung region is directly proportional to the ventilated lung volume viewed

by the scintillation detector. Comparisons between different lung regions can therefore be made using isotope techniques. Sutherland et al (103) express the components of lung volume both as a percentage of regional TLC and as a regional alveolar volume related to TLC alveolar volume (Fig. 3).

Conventional determination of lung volumes had shown that RV/TLC ratios increased with age, but prior to the work of Jones et al (104) it was unclear whether this was due to focal or generalised changes in the lung. Figure 4 summarises the results of Jones et al, demonstrating a uniform increase in RVr/TLCr throughout the lungs with increasing age. It was also seen that, despite increasing age, the ratio of upper to lower zone RVr/TLCr values did not change significantly (Fig. 5). These uniform changes in RVr/TLCr probably reflect a generalised reduction in lung elastic recoil with aging, leading to premature airways closure at a higher lung volume than during youth. A similar explanation has been offered to explain why closing capacity approaches FRC with increasing age (78,79).

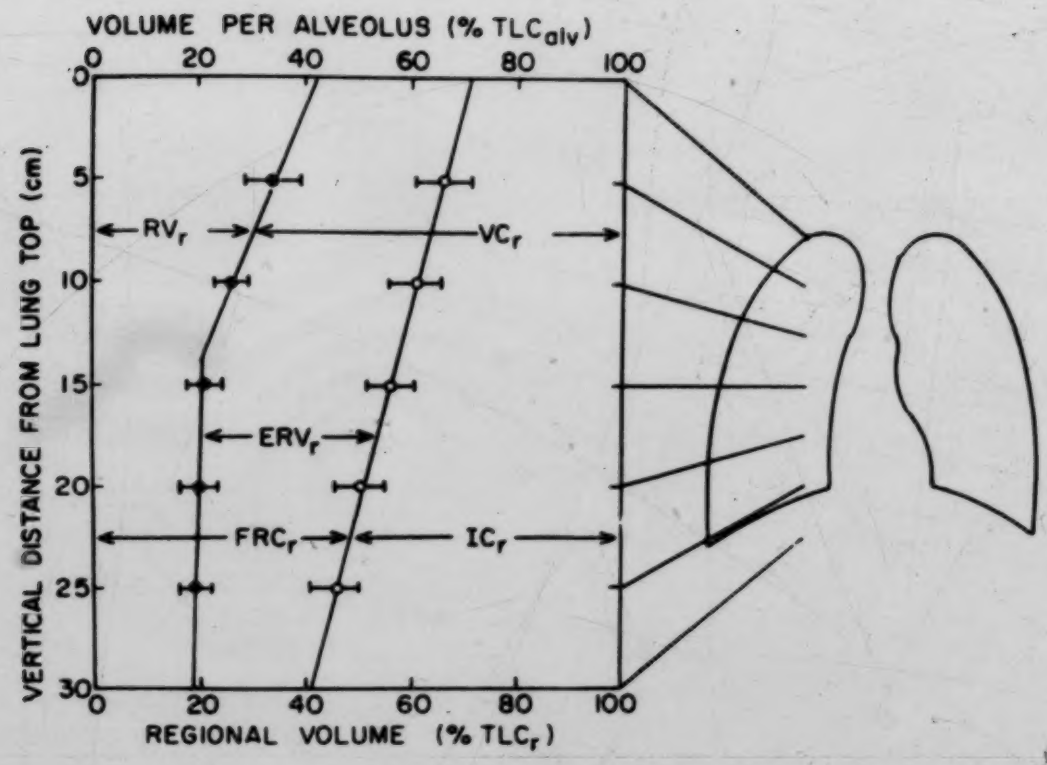


Fig. 3 - Regional subdivisions of lung volume in eight seated normal subjects. Solid and open circles represent results obtained at RV and FRC respectively. Bars indicate ± 2 SEM.

Data from Sutherland et al (103) as redrawn by Millic-Emili in Sem. Nucl. Med. 1971; 1:246. With permission from the author and publisher.

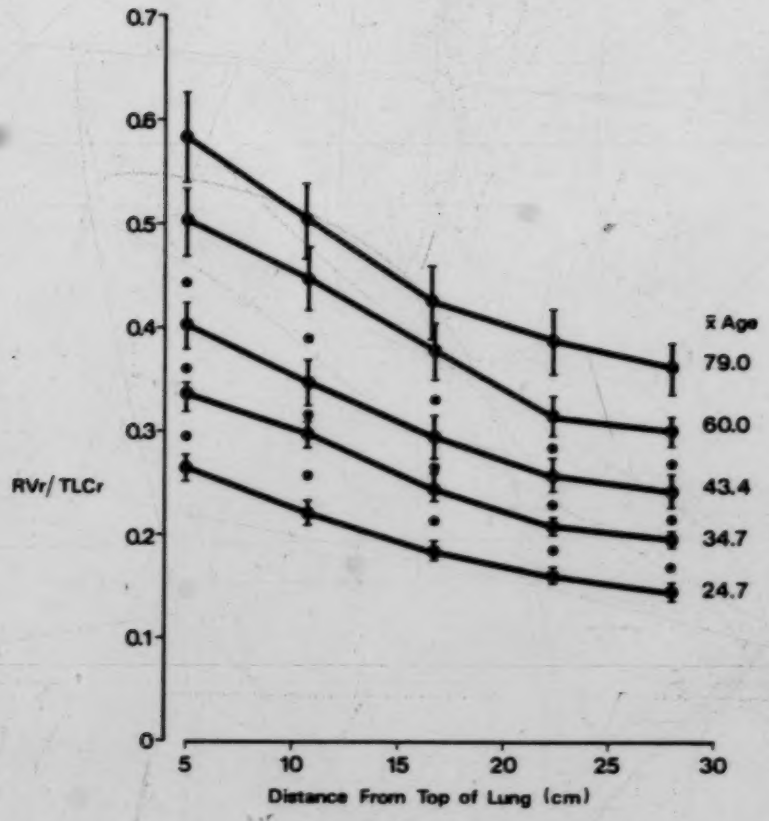


Fig. 4 - Variation in RVr/TLCr with age. Results shown as mean ± 1 SEM in 5 lung regions at ages indicated to right of each line. Significant differences ($P<0.05$) exists between specified regions (*) of adjacent age groups.
With permission from J. Appl. Physiol (104).

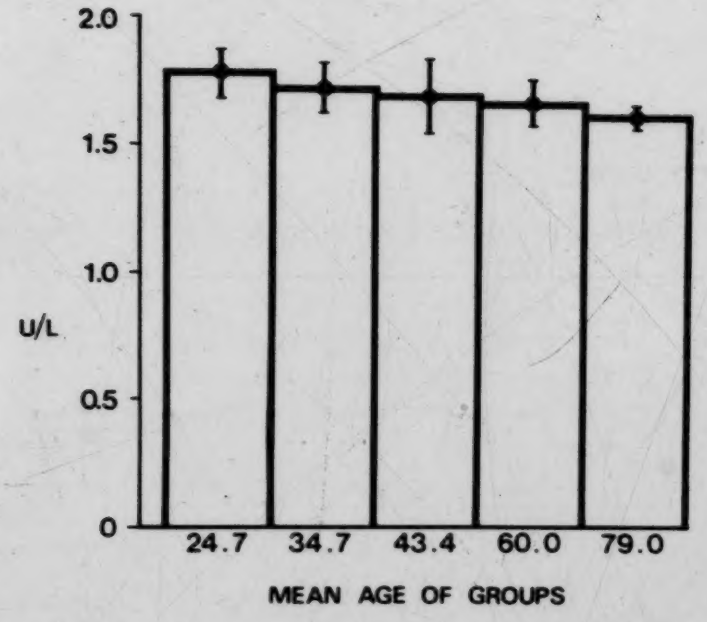


Fig. 5 - Ratio of upper (zones 1 and 6) and lower (zones 5 and 10) RVr/TLCr for different age groups expressed as mean ± 1 SEM. No statistically significant difference existed between age groups.
With permission from J. Appl. Physiol. (104).

Tests of overall lung function frequently show evidence of airflow obstruction in elderly smokers, particularly when assessed by single breath N_2 washout or maximum expiratory flow: volume techniques (105). There is a steady fall in maximum expiratory flow rates with age, this deterioration being accelerated by smoking (106). The importance in detecting early deterioration is emphasised by Fletcher and Peto who have shown that the accelerated rate of

deterioration of lung function in smokers reverts to a normal rate within a few years after stopping smoking (106).

Even when closing volume and other tests of small airways function are normal in young smokers, there may be an increase in RVr/TLCr ratios. The changes were predominantly in the lower lung zones, with no evidence of airways disease in the upper zones, when assessed in seated subjects (97). This is shown in figure 6, the mean age of both smokers and nonsmokers represented here being 23.8 years. Using a supine moving scanner technique Seaton and Ogilvie had found that ventilation in the upper lung zones was significantly decreased as compared to normals in a group of asymptomatic smokers (96). The average age of the subjects in their study was older (41 years), but the reason for the difference in findings was almost certainly the different posture of the subjects in the two studies (97,98). Thus the scanning technique employed, as discussed in the preceding section, has significant influence on the pattern of regional ventilation or perfusion results recorded.

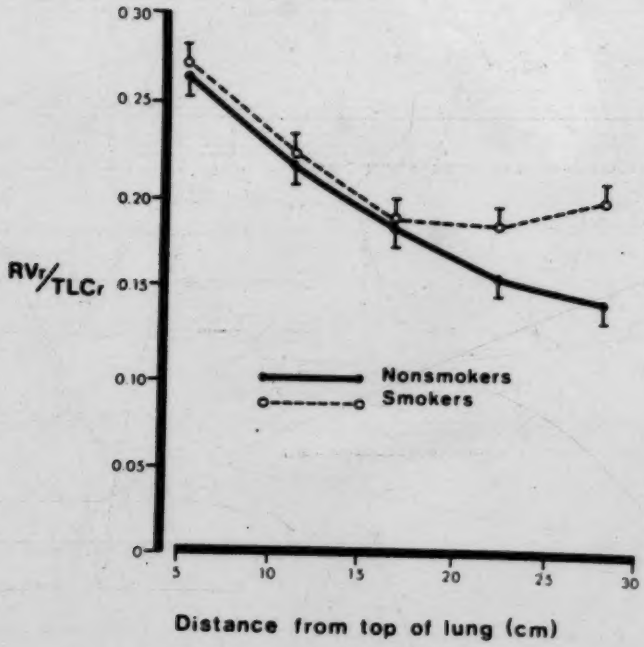


Fig. 6 - RVr/TLCr for different lung regions for 20 smokers and 11 non-smokers with mean age 23.8 years. Results are expressed as mean ± SEM.
With permission from Chest (97).

Increased airways resistance has been shown to influence the uniformity of lung ventilation, this being further affected by changes in the compliance of the alveolar units (107). As inspiratory flow rates increase with higher respiratory frequencies the contribution of airways resistance to the homogeneity of lung ventilation becomes increasingly important. In a recent study, breathing at 60 breaths per minute was shown to significantly impair regional ventilation in patients with obstructive lung disease when compared to the measurements made at 10 breaths per minute. The higher respiratory frequency did not affect the pattern of regional ventilation in normals (108). The changes observed in asthmatic subjects studied varied in severity between different lung zones, the greatest deterioration being observed in those regions presumed to have highest airways resistance and hence highest regional time constants (108,109). Bronchodilator administration (Isoproterenol delivered by a Bird nebuliser) was shown to significantly improve the pattern of regional ventilation, as well as preventing the deterioration previously observed during hyperventilation (Fig. 7).

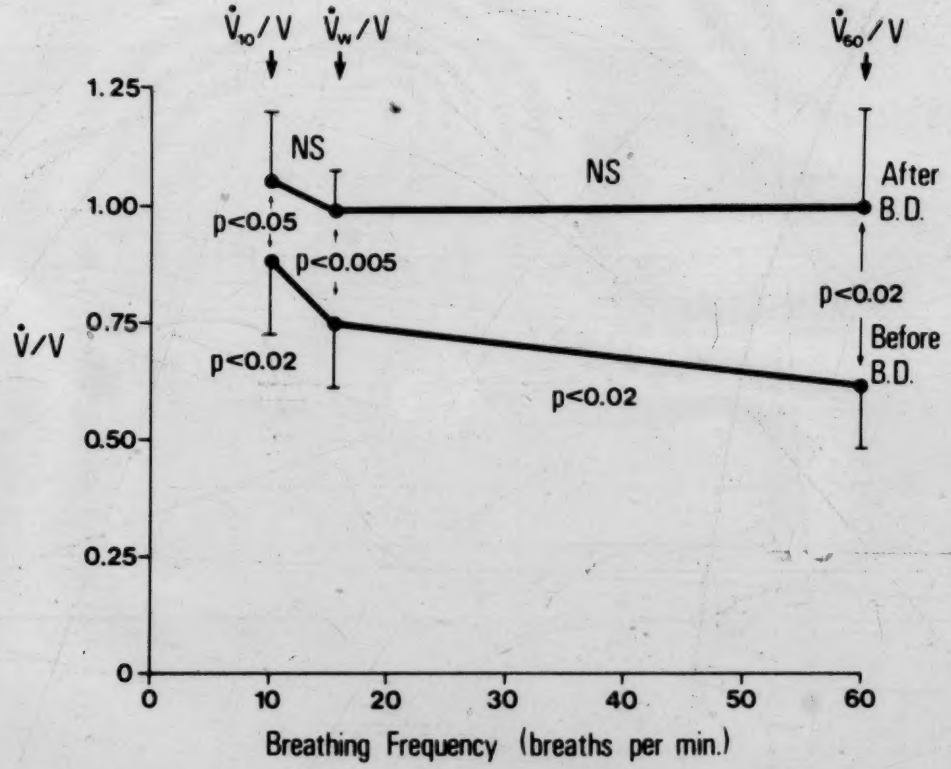


Fig. 7 - The effect of breathing frequency on ventilation, both before and after bronchodilator (BD), in lung regions of asthmatics shown to have greatest frequency - dependent reduction in ventilation. Breathing frequency during measurement of washin ventilation ($\dot{V}w/V$) was 15.5 breaths per minute before and 15.8 breaths per minute after BD. $\dot{V}_{10}/V$ and $\dot{V}_{60}/V$ denotes ventilation measurements per unit volume at 10 and 60 breaths per minute respectively. Values expressed as mean ± 1 SEM.

Reproduced with permission from J. Appl. Physiol. (108).

d) The effect of increased bronchomotor tone on regional residual volume

Asthma has been shown to be associated with a patchy change in regional lung function (108,109). Heckscher et al (11) studied 10 asthmatic subjects who were clinically in remission, 4 of these having normal regional ventilation distribution at the time of study. Two of their subjects had marked derangement of regional ventilation in all lung zones, whilst the other 4 had greater disturbance at the lung bases with the mid zones least affected. The hypoventilated regions were also found to have diminished perfusion, with low ventilation to perfusion ratios. Eight of their subjects had been studied 5 years previously and the degree of deterioration in regional lung function was found to correlate with the general change in overall lung function.

Heckscher studied all his patients in the supine position. Studying seated subjects, it has also been shown that the basal lung ventilation is most severely affected in chronic bronchitics, asthmatics and smokers (97,108). This is not suprising since airways closure begins in the most dependent lung regions as RV is approached, subsequently progressing up the lungs (77). Siegler et al (12) showed that the pattern of airways closure was more uniformly distributed from lung base to apex in asymptomatic asthmatics. He also found a reduced vertical gradient of regional residual volumes due mainly to increased RVr/TLCr

in the lower zones.

If a subject inhales slowly from RV, a bolus of ^{133}Xe inhaled at the start of inspiration will be preferentially distributed to the lung apices, the pattern of airways closure from base to apex explaining this observation (110). Using this technique Engel et al (8) studied a series of 7 seated normal subjects before and after methacholine inhalation. As in the asymptomatic asthmatics reported by Siegler (12), the vertical gradient of regional residual volumes was found to be decreased after methacholine, due to increased lower zone RVr. The changes were reversed by bronchodilator (Isoprenaline). Reduction in bronchomotor tone, by inhaling Isoprenaline, was also found to significantly affect the pattern of regional distribution of the inhaled ^{133}Xe bolus, the upper to lower distribution ratio increasing from 3.23 ± 0.47 (before) to 5.49 ± 0.85 (after bronchodilator). They suggest that methacholine exerts its effect by altering the critical opening or closing pressures of the airways. Increased bronchomotor tone therefore results in a larger proportion of closed airways and a wider range of critical opening or closing pressures (Fig. 8).

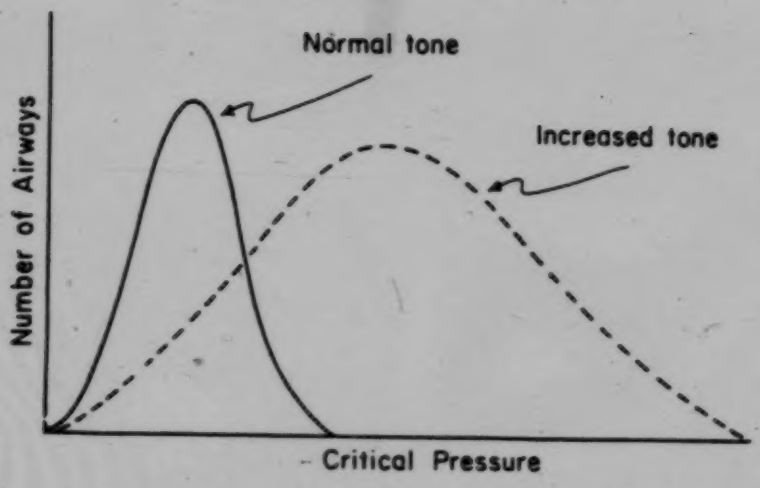


Fig. 8 - Diagrammatic representation of relative distributions of critical opening or closing pressures in the presence of normal bronchomotor tone and during bronchoconstriction. Assuming identity of pleural and extrabronchial pressures, abscissa reflects transpulmonary pressure with RV at the origin. (The number of closed airways in a lung region ordinarily increases down the lung, also depending on the lung volume at which measurements are made).
Reproduced with permission from J. Appl. Physiol. (8).

A further recent study in mongrel dogs, in whom bronchoconstriction was induced by methacholine, has also supported the concept of an increased range of critical opening pressures in the presence of increased bronchomotor tone (111). Units with a high critical opening pressure will open late in inspiration. These units will also tend to close prematurely at higher than usual lung volumes.

Results from animal studies of bronchoconstriction must, however, be applied with caution to humans, since the mechanics of lung function in dogs are different from in man. Collateral ventilation is much more important in dogs than man and the gradient of RVr and the pattern of airways closure also very different (73).

The determinants of regional lung function are clearly multifactorial, the relative importance or severity of coexistent factors determining the ultimate function. The effect of age on alveolar compliance and the pattern of airways closure affects everyone. Increased, or decreased, bronchomotor tone and other lung diseases (in particular irreversible airways obstruction) are also major determinants of regional residual volume and ventilation. The pattern of breathing, especially respiratory frequency, affects regional ventilation, as well as being important in the distribution of aerosolised drugs used in clinical investigation or therapy. The posture of the subject must always be taken into account, apical lung perfusion and ventilation being much less when erect than when supine. Finally the sensitivity and limitations of the equipment used must not be forgotten when interpreting the results of regional lung function, whether in health or disease.

Chapter III

EXPERIMENTAL

(i) Patient selection criteria

Subjects were obtained for the study either as healthy volunteers or by specific referrals from physicians within the Pulmonary Division of the University of Alberta Hospital. Those referred for challenge testing had symptoms such as chronic cough, intermittent dyspnea, episodic wheeze or chest tightness for which no cause had been identified by routine assessment techniques. No subjects with documented asthma or chronic lung disease of other etiology were included in this series. The criteria for inclusion of referred subjects in the study group were therefore:

1. Nonsmoker
2. Unexplained respiratory symptoms
3. Normal physical examination
4. Normal chest radiograph
5. Normal pulmonary function test results

All volunteers were healthy, with no preceding respiratory symptomatology. Physical examination and chest radiography were not performed on these volunteers.

Altered airways sensitivity to methacholine (and also to other inhalational challenge agents) has been reported following recent viral infections (56,57) as well as associated with ingestion of several medications (Table 1). Care was therefore taken to minimise the influence of these factors. Medications known to influence airways reactivity were discontinued prior to performing the challenge test in accordance with the ATS recommendations (35) (Table 2).

Table 1

Factors affecting airways sensitivity to inhaled methacholine (7,25,31,32,39,43,44)

Increased responsiveness	Decreased responsiveness
Suggestion	Suggestion
Acute viral respiratory tract infections	Bronchodilators
	Antihistamines
Influenza vaccination	Natural Rubeola
Recent antigenic challenge	Cromoglycate
Recent Rubeola vaccination	Steroids
Air pollution	Atropine

Table 2

Recommended ideal time interval between last medication and bronchial challenge (35)

Drug	Time interval (hours)
Inhaled bronchodilators	
Isoproternol	4
Terbutaline	12
Salbutamol	12
Atropine and analogs	10
Oral bronchodilator	
Short acting theophyllines	18
Long acting theophyllines	48
Antihistamines	48
Cromoglycate	48

(ii) Summary of protocol

1. Obtain informed written consent
2. Pulmonary function tests
3. Measure RVr/TLCr
4. Methacholine challenge test
5. Repeat RVr/TLCr
6. Measure FVC, FEV_1, $\dot{V}_{50}$, $\dot{V}_{75}$
7. Administer bronchodilator
8. Repeat FVC, FEV_1, $\dot{V}_{50}$, $\dot{V}_{75}$ at 5 and 10 min. post bronchodilator
9. Repeat RVr/TLCr (once spirometry normal).

This sequence takes approximately 2 hours to complete. If full pulmonary function assessment had been performed within the week prior to testing (without intervening illness or medication) only a flow-volume loop, FVC, FEV_1 and closing volume measurement were repeated on the day of challenge testing.

(iii) Pulmonary function assessment

a) Flow-Volume Loop

This test was performed particularly to determine flow rates at low lung volumes as a test for early small airways disease (107,112,113). The maximum expiratory flow rate (MEFR) and forced vital capacity (FVC) are both affected by the amount of voluntary effort during performance of spirometry. The terminal portion of the flow-volume loop, however, is relatively effort independent, being

reproducible with both different levels of expiratory effort from TLC and for forced expiration from different lung volumes (Fig. 9).

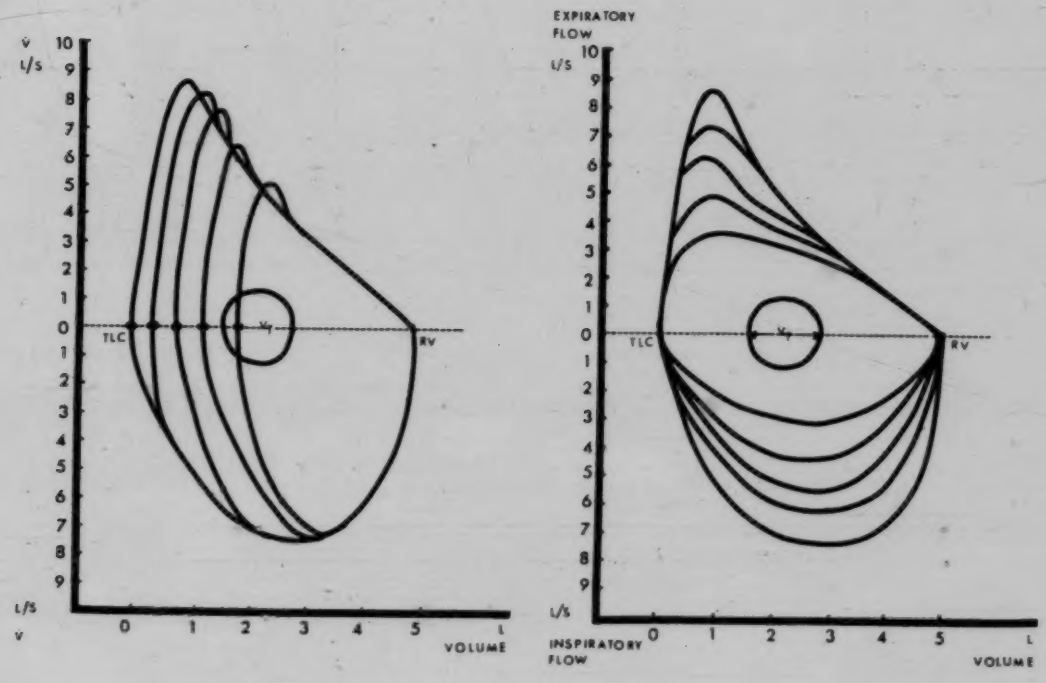


Fig. 9 - The effect of different starting volumes (left) and different levels of effort (right) on the latter "effort-independent" portion of the flow-volume loop (Results of subject 23)

The MEFR is achieved with forced expiration from TLC; when the starting lung volume is less, the maximum expiratory flow rate is also reduced due to increased airways resistance (since the airways diameter is less at

lower lung volumes) (107). The significance of such changes is magnified in the presence of disease states such as asthma or chronic lung diseases which affect the airways mucosa: in asthma due to mucosal edema and due to viscid secretions in chronic bronchitis, for example. With pulmonary fibrosis the opposite of this effect is observed, the airways being more rigid and pleural pressure more negative. The airways are therefore held open despite reduction in lung volumes, resulting in higher than normal flow rates at low lung volumes.

Method

A noseclip is applied and the subject breathes through a large aperture rubber mouthpiece and three-way valve. The valve is initially open to room air. During normal tidal breathing the valve is turned at FRC, connecting the subject into the recorder system (Ohio 842 Spirometer). A tidal breath tracing (V_T, Fig.10) is then recorded using an X-Y pen plotter (Hewlett-Packard 7046A X-Y recorder), expiratory flow rate (ℓ/sec) plotted on the Y axis against lung volume (ℓ) on the X axis. From FRC the subject is now asked to take a maximal inspiration to TLC, then perform a maximal forced expiration down to RV, at which point he takes a maximal inspiration back to TLC giving the maximum inspiratory flow rate (MIFR). The procedure is repeated and the best tracing (i.e. the largest FVC without coughing) used to calculate expiratory flow rates at 50% ($\dot{V}_{50}$) and 75% ($\dot{V}_{75}$) of FVC (Fig. 10).

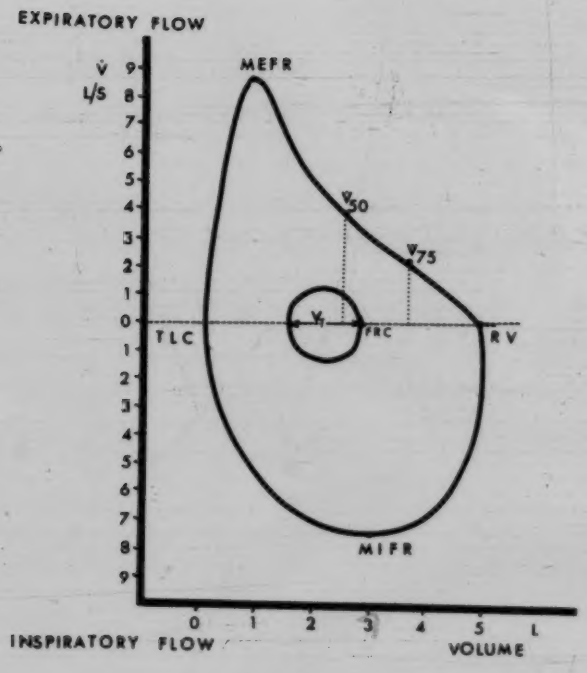


Fig. 10 - Flow-volume curve measurements (Subject 23)

b) Isovolume flow rates

For routine purposes the above method is used for determining $\dot{V}_{50}$ and $\dot{V}_{75}$. When, as during inhalational challenge testing, there is an alteration in vital capacity, it is difficult to compare these parameters directly. To overcome this the $\dot{V}_{50}$ and $\dot{V}_{75}$ are recalculated at standardised lung volumes, i.e. at 50% and 75% of control FVC (114). The assumption in the calculation of isovolume

flow rates is that TLC alters very little, reduction in FVC during challenge testing being due to increased RV. It can be seen in the example below (Fig. 11) that, if $\dot{V}_{50}$ and $\dot{V}_{75}$ are calculated on the basis of individual FVC manoeuvers, they appear only slightly reduced, especially $\dot{V}_{75}$. If recalculated as isovolume flow rates, the outer tracing representing control values, then $\dot{V}_{75\ isovol}$ is now seen to be 0 ℓ/sec and $\dot{V}_{50isovol}$ also greatly decreased after methacholine inhalation.

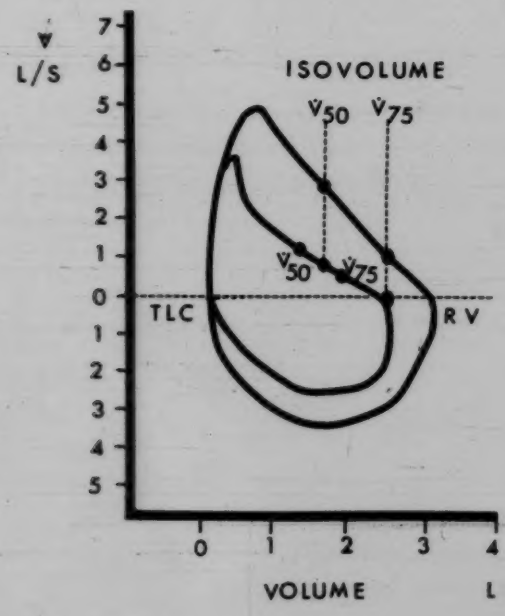


Fig. 11 - Isovolume flow rate determination (Subject 5). The outer curve shows the control $\dot{V}_{50}$ and $\dot{V}_{75}$, with the inner curve showing the flow-volume loop after inhaling 5 mg/ml methacholine sclution. Note the relative positions of the $\dot{V}_{50}$ and $\dot{V}_{75}$ as measured on the post-methacholine curve, compared to the isovclume flow points.

c) Single breath N_2 washout (closing volumes)

Small airways function as well as the overall uniformity of lung ventilation can be assessed by this technique (77-82). The principle underlying this test is that the pleural pressure increases from around -10 cmH_2O at the apices to about -2.5 cmH_2O at the lung base. This means that the transpulmonary pressure (airway pressure minus pleural pressure) is greater at the top of the lungs and the distending force on the alveoli is consequently greater at the apices than in the basal lung regions. As lung volume approaches RV during expiration, the pleural pressure become less negative which, associated wdith decreasing alveolar volumes and reduced airways diameters, results in airways closure. The airways at the lung bases close first but, at RV, there are closed airways in all lung zones, although the greatest percentage of closed airways are at the bases. The volume at which closure first occurs is the closing volume (%VC) or the closing capacity (%TLC).

Method

First a calibration mark is made on the Y axis of an X-Y recorder (Hewlett-Packard 7046A X-Y reocrder) for N_2 concentration in room air (79%). Next a noseclip is applied and the subject breathes room air through a rubber mouthpiece and 3-way valve. He is instructed to exhale down to RV, at which point the valve is turned to permit a vital capacity breath of 100% O_2. A second valve is now turned and he slowly exhales into a spirometer (Ohio 842

spirometer) past an N_2 analyser (Ohio 700 N_2 analyser), the rate of exhalation being no more than about 0.5 ℓ/sec. The N_2 concentration is recorded continuously as a percentage concentration in expired air on the Y axis against volume exhaled on the X axis.

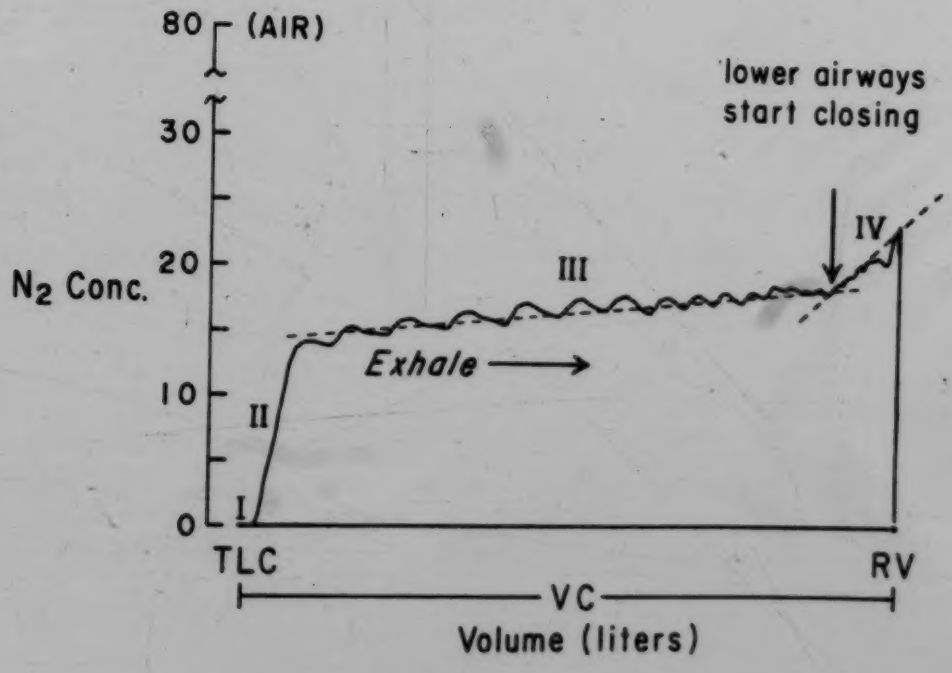


Fig. 12 - Single breath N_2 washout

Fig. 12 shows a typical N_2 washout curve obtained by this technique. Initially (Phase I) there is no N_2 at all whilst dead space gas (both major airways and equipment dead spaces) containing only 100% O_2 is exhaled. The abrupt rise in N_2 concentration (Phase II) reflects the start of

exhalation of alveolar gas, reaching a plateau (Phase III). In a slow steady exhalation, Phase III demonstrates oscillation due to cardiac pulsation; if these oscillations are absent the exhalation has been too rapid.

If ventilation throughout the lungs was completely uniform, the N_2 concentration would also be uniform and the Phase III plateau horizontal. Since Phase III slopes upwards, this means that the exhaled gas composition is constantly changing due to different rates of alveolar emptying. The alveoli contributing to the exhaled gas have different N_2 concentrations and, since those having lowest N_2 concentrations (at the lung bases) empty most rapidly, Phase III slopes upwards. At the point where basal airways begin to close there is a sudden rise in N_2 concentration (Phase IV) as gas is now being exclusively exhaled from the upper lung zones - i.e. the alveoli and airways which were patent at RV and had therefore received least O_2.

The closing volume is calculated as a percentage of VC. The closing capacity may be obtained by adding the CV (in liters) to the RV determined by helium dilution. The slope of Phase III is measured as $\%N_2$ change per liter expired air.

The closing capacity increases with age, approaching FRC by a mean age of 66 years (79) probably due to reduced elasticity of lung tissue. Narrowing of the peripheral bronchioles also leads to earlier airways closure, whether due to secretions (chronic bronchitis), inflammation

(asthma) or fibrosis. This has been shown to be a sensitive test to demonstrate early small airways disease and may be abnormal before other tests are affected.

d) Lung volumes

Two methods of measuring the functional residual capacity (FRC) are employed. The helium dilution method measures only ventilated FRC, whereas total FRC, including trapped air, may be determined in the body plethysmograph.

FRC (Helium)

The dead space of a closed circuit spirometer (Godart Pulmotest) is first calculated by adding a volume (V_1) of known concentration (C_1) helium gas to the system. After equilibration the concentration of helium (C_2) is again recorded (determined with the Godart FRC He computer). The spirometer deadspace (V_s) of this apparatus is generally 9.5 ℓ, but is confirmed by the following calculation:

$$C_1V_1 = C_2V_2 \qquad \text{where } V_2 = (V_1 + V_s)$$

$$\text{Hence} \qquad V_s = \frac{(C_1-C_2)\cdot V_1}{C_2}$$

The helium is now evacuated from the circuit and a volume of helium added to bring the spirometer to a known volume (V_3) and allowed to equilibrate, achieving a concentration (C_3, Fig. 13). The seated subject, wearing noseclips and breathing through a rubber mouthpiece and 3-way valve, is connected into the spirometer circuit at the

end of a tidal breath (i.e. at FRC). Carbon dioxide is removed and oxygen added to maintain a constant spirometer volume and to prevent hypercapnia or hypoxia. A couple of deep breaths speed equilibration to a new helium concentration (C_4, Fig. 13). Since the patient was introduced into the spirometer at FRC, the initial helium concentration (C_3) is now diluted by the volume of air present in the lungs at FRC.

$$C_3V_3 = C_4V_4 \qquad \text{where} \quad V_4 = V_3 + FRC \qquad \text{and} \quad V_3 = V_s$$

$$\text{Hence} \quad FRC = \frac{(C_3-C_4)\cdot V_s}{C_4}$$

The accuracy of this method of FRC calculation depends on the subject being turned into the spirometer circuit exactly at FRC.

Synchronised respiration

The ability to maintain synchronised respiration with a metronome at 30 breaths per minute is determined during tidal breathing. The amount by which the FRC increases from baseline tidal breathing is measured (ΔV, Fig. 13), this being required for the subsequent body box FRC determination.

Fig. 13 - Spirometric determination of lung volumes and flow rates (Subject 23).

C_3He = initial [He]

C_4He = equilibrated [He]

A = 2 deep breaths to speed equilibration

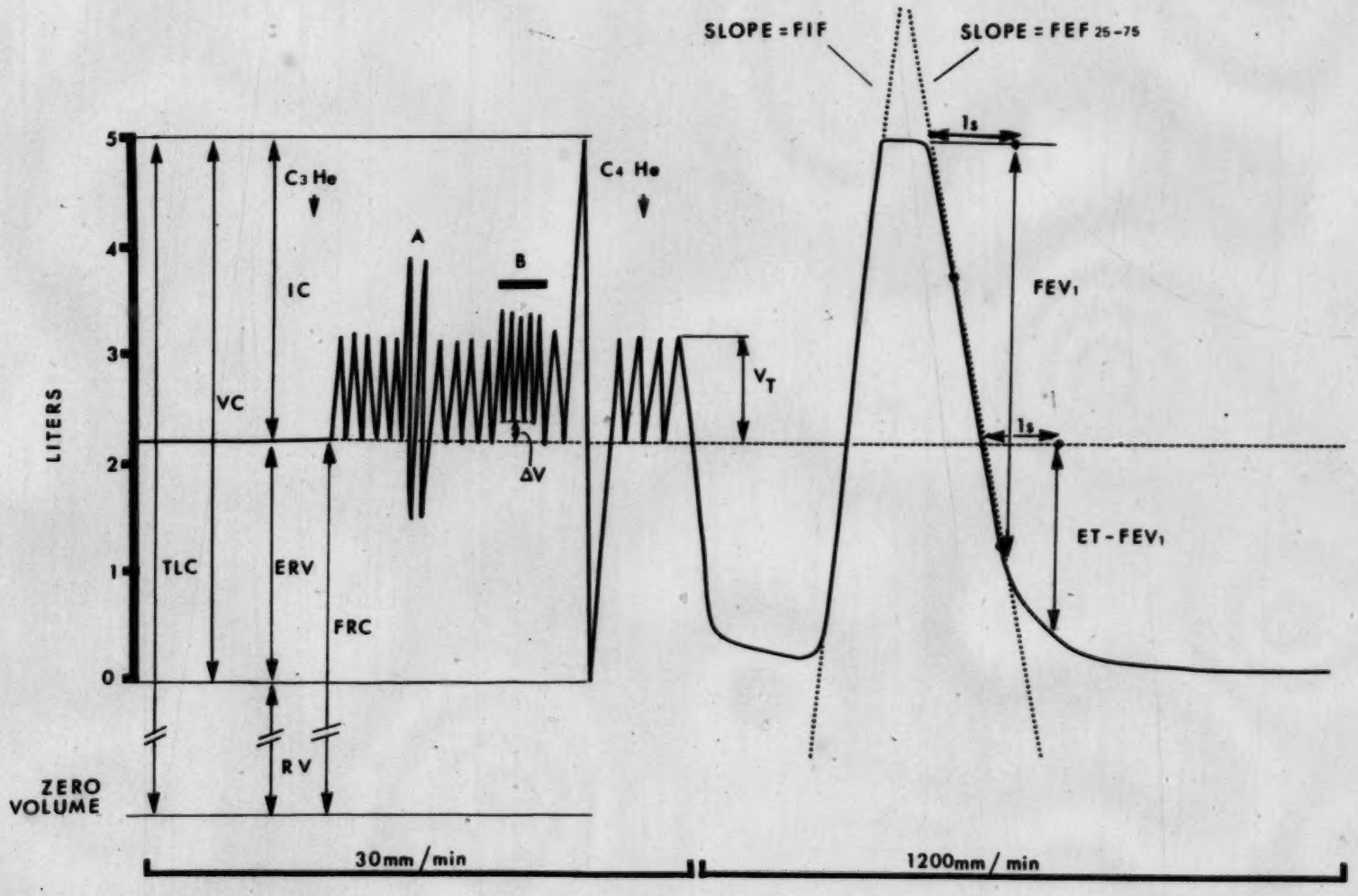
B = breathing synchronised with metronome, 30 breaths/min

ΔV = change in FRC during synchronised respiration

FEF_{25-75} - The forced expiratory flow rate between points shown at 25% and 75% of forced vital capacity.

FEV_1 - The volume exhaled from TLC in 1 second during a maximal expiration.

$ET\text{-}FEV_1$ - The volume exhaled below FRC in 1 second when measured during a maximal forced expiratory maneuver.


SLOPE = FIF
SLOPE = FEF 25-75
1s
C3 He
C4 He
A
B
IC
VC
FEV1
VT
1s
ΔV
LITERS
5
4
3
2
1
0
TLC
ERV
FRC
ET - FEV1
RV
ZERO VOLUME
30mm / min
1200mm / min

VC, FEV_1, FIF and derived data

After FRC determination, the subject is now instructed to inhale fully to TLC, then exhale slowly to RV. The spirometer recording speed is now increased to 1200 mm/min and the subject exhales to RV, then inhales rapidly to TLC. The slope of this tracing (Fig.13) gives the forced inspiratory flow rate (FIF). From TLC he performs a rapid forced expiration until unable to exhale further (i.e. to RV). Several lung volumes can now be measured from this tracing (Fig. 13). The helium dilution RV is calculated from FRC(He) - ERV.

e) Body plethysmograph

FRC determination

The body box method of measuring FRC determines total FRC, including both ventilated and unventilated (trapped air) regions. The method of DuBois et al is used (115).

Method

The subject sits in the body box (Siemens Siregnost FD40, with MFE 815M Plotamatic X-Y recorder) with the door sealed and the box valve open to the atmosphere. After allowing adequate time for temperature equilibration in the box, the subject applies noseclips and breathes through a wide-bore rubber mouthpiece. The box valve is now closed to the atmosphere. Airways pressure is recorded at the mouth and fed to the Y axis of the pen recorder; internal box pressure is recorded simultaneously against this on the X

axis. The subject breathes synchronously with a metronome set at 30 breaths per minute.

At the end of a tidal expiration a shutter at the mouth is closed, but the subject instructed to continue to attempt to inhale and exhale in time with the metronome. The deflections recorded on the X-Y plotter (Fig. 14) show the relative change in alveolar (mouth) pressure with box pressure. From Boyle's Law for a closed system, the pressure and volume relationship is constant, pressure being inversely related to volume.

$$\text{i.e. } P.V = K$$

$$\text{or } \quad P \propto \frac{1}{V}$$

In the body box the ΔP_{box} therefore reflects the changes in chest size (ΔV_{thorax}) during attempted breathing against the closed shutter. At FRC there is zero airflow. Hence, at FRC, $P_{alveolar} = P_{box} = P_B$

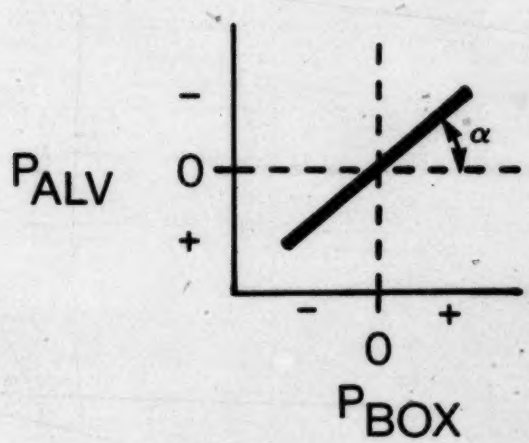


Fig. 14 - Determination of α angle from body box tracing.

The cotangent of the angle α (Fig. 13) gives the slope of the line.

$$\therefore \text{Cot } \alpha = \frac{\Delta P_{box}}{\Delta P_{alv}} \equiv \frac{\Delta V_{thorax}}{\Delta P_{alv}}$$

The polarity of the leads to the X and Y axes are now reversed and the mouth shutter closed at the end of a normal tidal inspiration. A tracing is again obtained of ΔP_{alv} versus ΔP_{box}, and the α angle again determined so that the mean α of the two methods may be determined for R_{aw} calculation. The α angle obtained at end-expiration is used to obtain FRC.

From Boyle's Law, $P_1V_1 = P_2V_2$

$$= (P_1 + \Delta P)(V_1 + \Delta V)$$

$$= P_1V_1 + P_1\Delta V + V_1\Delta P + \Delta P\Delta V$$

where $P_1 = P_B$

V_1 = Lung volume at FRC

$P_2 = P_1 + \Delta P$ (during attempted insp. or exp.)

$V_2 = V_1 + \Delta V$ (during attempted insp. or exp.)

Rearranging,

$$P_1V_1 - P_1V_1 - V_1\Delta P = P_1\Delta V + \Delta V\Delta P$$

$$-V_1\Delta P = \Delta V(P_1 + \Delta P)$$

$$-V_1 = \frac{\Delta V}{\Delta P}\ (P_1 + \Delta P)$$

But ΔP is very small in comparison with P_B and may be disregarded; also a negative FRC cannot exist.

$$\text{So} \quad FRC = V_1 = \frac{\Delta V}{\Delta P} \cdot P_1$$

$$\text{or} \quad FRC = P_B \cdot \text{Cot } \alpha$$

The box is calibrated whilst empty, so the body volume in liters of the subject (weight,W, in kg ÷ 1.1) must be subtracted from the empty box volume (890 ℓ) to give the actual volume during the test. If this were not done the calibration would be incorrect, the change in box pressure per unit volume being greater with a subject inside than with the box empty.

X axis, volume calibration = 40 ml/cm

Y axis, pressure calibration = 7.35 mmHg/cm

$\therefore \frac{\Delta V}{\Delta P}$ calibration $= \frac{40}{7.35} = 5.42$ ml/mmHg

Correction factor for subject's volume $= \frac{890-(W \div 1.1)}{890}$

The final formula now employed only requires entry of body weight (kg), barometric pressure (mmHg) and the α angle to allow FRC to be calculated at BTPS:

$$FRC = (P_B-47)\cdot \text{Cot}\ \alpha \cdot \left(\frac{890-(W \div 1.1)}{890}\right)\cdot 5.42\ \ell$$

The tendency for the FRC baseline to rise when breathing in time with the metronome has previously been determined during the spirometric lung volume measurements. Any volume change demonstrated is now subtracted from the FRC value obtained above to calculate true total FRC.

The volume of trapped air may now be calculated by [FRC (box) - FRC (He)].

Airway resistance

Instead of recording ΔP_{alv}, flow rate ($\dot{V}$) is recorded

at the mouth with a pneumotachograph and plotted on the Y axis against ΔP_{box} on the X axis (116).

$$R_{aw} = \frac{\text{Pressure difference down airway}}{\text{Flow rate in airway}} = \frac{P_B - P_{alv}}{\dot{V}}$$

If airways resistance is high, then a relatively greater effort per unit flow rate (i.e. ΔP_{alv}) will be required to overcome it, resulting in a larger ΔP_{box} deflection. The measurements are made during tidal breathing in time with the metronome. The result therefore gives the R_{aw} under "normal" breathing conditions, uninfluenced by large swings in intrathoracic pressure caused by forced expiration or inspiration.

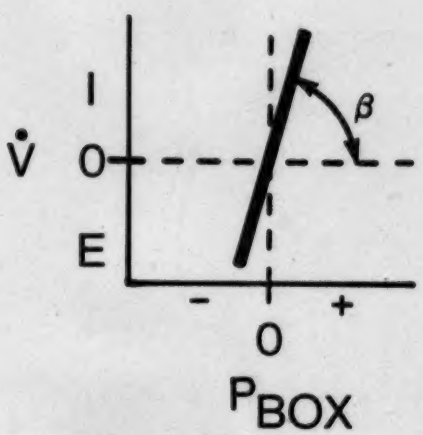


Fig. 15 - Determination of β angle for Raw.
I = Inspiration; E = Expiration

The relationship of $\frac{\Delta Palv}{\Delta Pbox}$ is given by the α angle previously calculated; the β angle (Fig. 15) gives the

relationship $\frac{\Delta Pbox}{\dot{V}}$.

$$\text{Tan } \alpha = \frac{\Delta Palv}{\Delta Pbox}$$

$$\text{Cot } \beta = \frac{\Delta Pbox}{\dot{V}}$$

$$\therefore \text{Raw} = \frac{\Delta Palv}{\dot{V}} = \frac{\Delta Palv}{\Delta Pbox} \quad \frac{\Delta Pbox}{\dot{V}}$$

Hence $R_{aw} = \text{Tan } \alpha \quad \text{Cot } \beta$

The pneumotachograph resistance is 0.3 $cmH_2O/\ell/s$ and the Y axis calibration for flow is 0.4 $\ell/s/cm$. Combining the calibration factors for the α and β angles:

$$\text{Calibration } \frac{Palv}{Pbox} = \frac{10 \text{ } cmH_2O/ml}{40 \text{ } ml/cm}$$

$$\text{Calibration } \frac{Pbox}{\dot{V}} = \frac{40 \text{ } ml/cm}{0.4 \text{ } \ell/s/cm}$$

$$\text{Hence, } \frac{Palv}{Pbox} \quad \frac{Pbox}{\dot{V}} = \frac{10 \text{ } cmH_2O/ml}{40 \text{ } ml} \quad \frac{40 \text{ } ml}{0.4 \text{ } \ell/s}$$

$$= 25 \text{ } cmH_2O/\ell/s$$

Thus the final equation used is

$$R_{aw} = (\text{Tan } \alpha \quad \text{Cot } \beta \quad 25) - 0.3 \text{ } cmH_2O/\ell/s$$

f) Diffusion Capacity

Carbon monoxide is chosen as the tracer gas for the diffusion capacity measurement due to its high affinity for hemoglobin. This permits rapid determination of the rate of diffusion across the alveolar-capillary membrane after a

single vital capacity breath of gas (117,118).

$$D_L CO = \frac{\text{Rate of CO uptake}}{\text{Alveolar Pco}}$$

This is accurate so long as the pulmonary capillary Pco is zero. A back diffusion pressure due to pulmonary capillary Pco (as from heavy smoking) produces an apparent reduction in $D_L CO$ unless this is taken into consideration.

$$D_L CO = \frac{\dot{V}_{CO}}{P_{Aco} - P_{\bar{C}co}}$$

$\dot{V}co$ = CO uptake

P_{Aco} = alveolar Pco

$P_{\bar{C}co}$ = pulmonary capillary Pco

Method

With noseclips applied the seated subject exhales to RV through a three-way valve (1, Fig.16). The valve is turned and he takes a vital capacity breath, to TLC, of a gas mixture containing known concentrations of CO and He (2, Fig.16). After a 10 second breathhold (3, Fig.16) he exhales rapidly (4, Fig.16) into a gas analyser (Medicraft Instruments Inc, NY, Automatic single breath diffusion apparatus; Diffusion test IIa, Instrumentation Associates, NY; Godart expirograph; Godart CO analyser; Godart FRC (He) computer). A sample of exhaled gas is collected shortly after the start of exhalation (i.e. after allowing for dead space gas to be exhaled) and analysed for [He] and [CO] (5, Fig.16). The breathhold time (BHT, Fig.16) is measured from a paper spirometer tracing, as is the breathhold volume (VC, Fig.16).

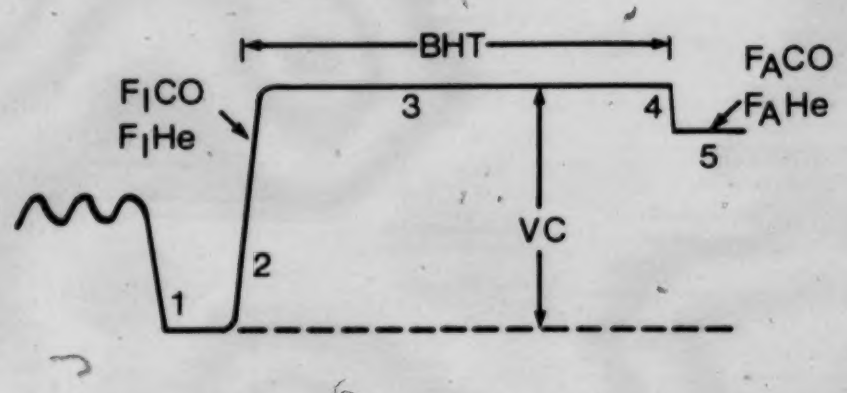


Fig. 16 - Diffusion capacity determination

The helium is present in the test gas to permit calculation of the alveolar volume, this being necessary since D_LCO increases with lung volume (119).

$$D_LCO = \frac{\frac{F_IHe}{F_AHe} \cdot VC \cdot STPD}{(P_B-47)\left(\frac{BHT}{60}\right)} \cdot \log_n\left(\frac{\frac{F_AHe}{F_IHe} \cdot F_ICO}{F_ACO}\right)$$

Where F_IHe is inspired [He]

F_AHe is alveolar [He]

F_ICO is inspired [CO]

F_ACO is alveolar [CO]

BHT is breathhold time (in seconds)

VC is vital capacity (in ml, measured from expirograph)

STPD is the correction factor for converting VC to STPD.

Predicted values for D_LCO are obtained from the formulae of Gaensler and Smith (Chest 1973; 63: 144):

Males: $[(0.00375)(V_ASTPD)] - [(0.153)(Age)] + 19.93$

Females: $[(0.00538)(V_ASTPD)] - [(0.083)(Age)] + 7.72$

V_A is alveolar volume at STPD (the numerator of the left hand part of D_LCO equation above) and Age is in years.

g) Bronchodilator response

After completion of the preceding tests, 2 metered doses of Salbutamol are administered to the subject. Ten minutes later body box R_{aw} measurements are repeated, followed by spirometric lung volume measurements and a flow-volume loop.

(iv) Xenon-133 Radiospirometry

a) Description of apparatus

The method chosen for this study employs the multi-detector system which has been in clinical use at the University of Alberta for the past 10 years (92-94). The system utilises 10 collinear pairs of detectors to provide anterior and posterior coverage of 5 regions of each lung (Fig. 17).

There are twenty 25.4 mm diameter, 12.7 mm thick Thallium-activated Sodium iodide crystals, each integrally mounted with a 25.4 mm photomultiplier. All detectors are shielded in 6 mm thick cyllindrical lead collimators, with

the detector face recessed 11.4 cm from the end of the collimator. The output from each collinear pair of detectors is fed through a common amplifier and analyser (with 70-90 KeV range). The mean (arithmetic) count is stored from each detector pair in computer memory. Storage of 200 frames of data, at 0.5 sec intervals, was used for each detector pair in this study (Fig. 18).

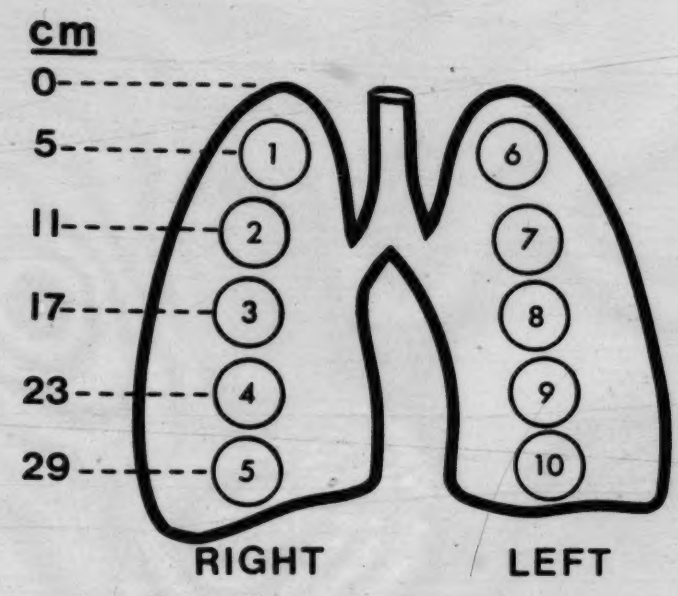


Fig. 17 - Diagrammatic representation of detector arrangement.

The collimators are mounted in anterior and posterior subframes around a chair. The anterior 10 detectors may be moved horizontally to accommodate patients with different thoracic dimensions. Both anterior and posterior detector banks may also be raised or lowered simultaneously to adjust

for different subject heights. The upper detectors are positioned so that their centers are approximately 5 cm from the top of the lungs, with 6 cm spacing between the centers of adjacent collimators over each lung. The centers of the highest and lowest detectors are therefore always 24 cm apart (Fig. 17). All posterior collimators are mounted such that their anterior faces, shielded by a sheet of perspex as a backrest, are in the same vertical plane. The anterior detectors are positioned on their subframe to correspond to the basic distribution of adult lung capacity: i.e. allowing more space between the collimator pairs at the lung bases than at the apices. It is also possible to adjust the lateral spacing of the detector banks over the two lungs, thus ensuring that the detectors are well centered on the appropriate lung zones for each subject. A seatbelt is fastened around the hips to help maintain the positioning of the subject throughout the testing.

Further details regarding the system specifications and computer assisted data storage are given by Friedenberg (92) and Filipow (120).

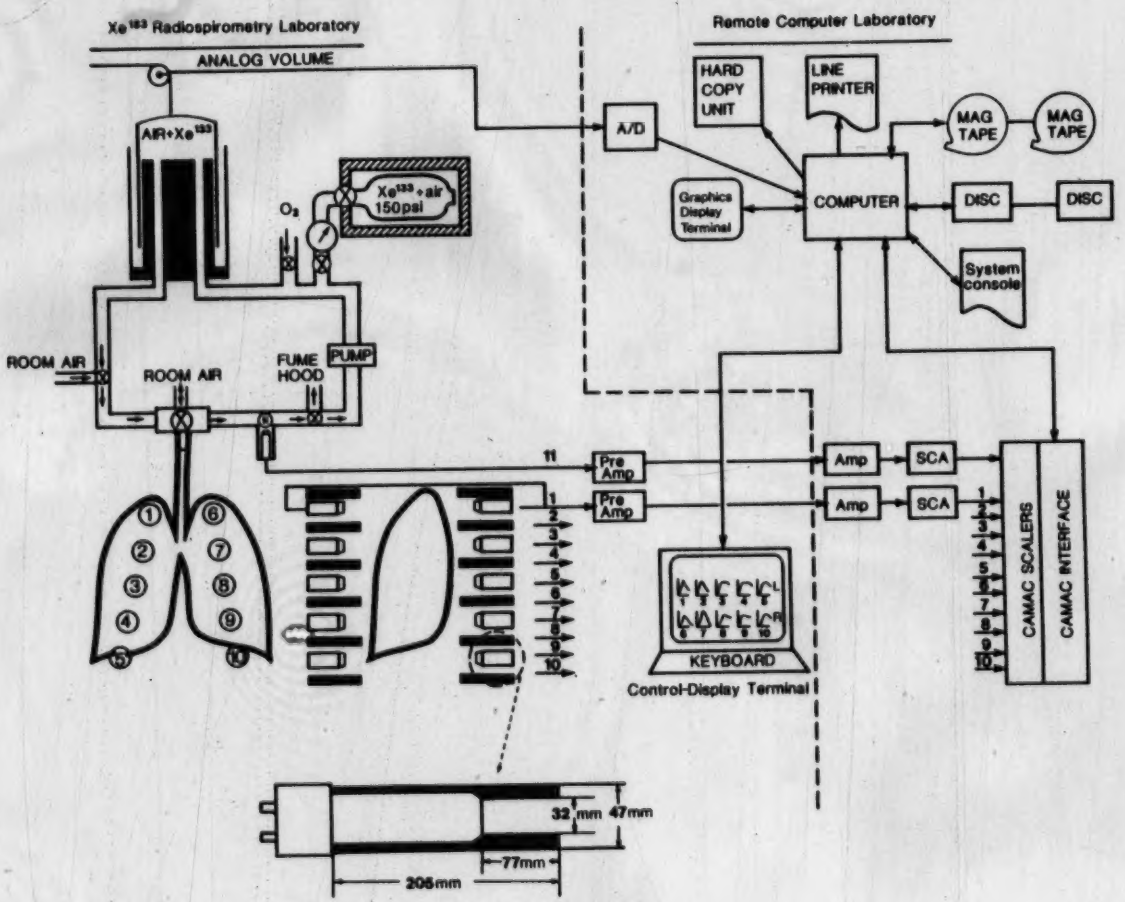


Fig. 18 - Instrumentation - Computer system for ^{133}Xe radiospirometry.

b) Dispensing ^{133}Xe

Xenon-133 is received as a gas in 5 ml glass ampoules containing approximately 1 Ci (37GBa) activity at 5 mmHg pressure. Figure 19 shows the dispensing system developed by Snyder and Overton (121) which permits easy division of the ^{133}Xe into aqueous and gaseous fractions.

Method

The stainless steel syringe, which will contain the aqueous solution, is autoclaved prior to use. The transfer piston is now withdrawn fully and the transfer volume, gas cylinder, syringe and chamber containing the sealed vial of ^{133}Xe are evacuated to about 10^{-2} mmHg. The transfer volume is then reduced to its minimum volume by moving the piston forward and all valves are closed. Next the vial is broken, valve 1 (Fig.19) opened and the transfer piston retracted fully, allowing more than 95% of the ^{133}Xe to enter the total transfer space of 1006 ml. Valve 1 is closed and, with valve 2 (Fig.19) opened, the transfer volume again reduced to its smallest value, thus allowing the desired fraction of the ^{133}Xe to enter the syringe. Valve 2 is closed and the syringe removed from the system to be filled with sterile, pyrogen-free water. The remainder of the ^{133}Xe in the system and vial is then transferred to the gas cylinder by repeated dilution of the gas in the vial by the transfer volume. The gas cylinder is then pressurised to 150 psi and attached, with appropriate lead shielding, to a spirometer through a pressure reducer and microleak valve.

At the end of the transfer process less than 1 mCi of ^{133}Xe remains within the dispensing unit. The radiation exposure to those dispensing the ^{133}Xe is minimal.

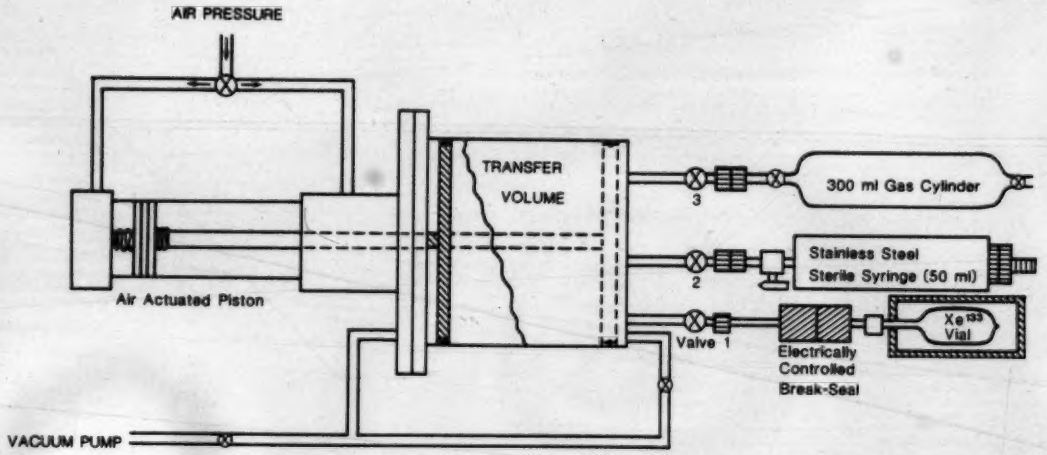


Fig. 19 - Dispensing system for ^{133}Xe.

c) Xenon-133 characteristics and dosimetry

The $T_{1/2}$ of ^{133}Xe is 5.27 days with decay to an isomeric state of ^{133}Cs by beta ray emission, subsequently either emitting a photon (81 KeV gamma radiation) or an internal conversion electron (30 KeV K X-radiation) before attaining its stable state of $^{133}_{55}Cs$ (122).

The test procedure involves equilibration with 0.5 mCi ^{133}Xe per litre room air in a rebreathing system, taking an average of 3 minutes before performing the Rvr/TLCr measurements. The subsequent washout phase from the lungs

is rapid in the absence of airways obstruction. Since the lungs have held ^{133}Xe for a few minutes, some has been carried by the blood stream and deposited in fat in the chest wall and other parts of the body. Washouts from these sites is slower due to the high affinity of ^{133}Xe for fat and also because it must first be carried back to the lungs by the bloodstream before it can be exhaled. The subject is equilibrated on the system 3 times during the study over a period of approximately 75 minutes.

The method of calculating the radiation dose has been documented in detail by Loken and Kush (122) and Goddard and Ackery (123). They have shown that the total lung dose is the product of the time to equilibration, the time at equilibration and the spirometer circuit ^{133}Xe concentration. The major dose is to the respiratory airways with a very small dose to the gonads (124). Thus 3 minutes rebreathing 0.5 mCi per litre ^{133}Xe gives a radiation dose to the lungs of 42 mRad with a gonadal dose of around 4 mRad (124), although others have calculated the dose to be much less (122). This compares with a single 100 kV_p PA chest radiograph delivering around 20 mRad to the chest and 0.7-15 mRad to the testes or 1.5-45 mRad to the ovaries (125). In contrast a plain AP abdominal radiograph gives 8.8 mRad to the testes and 357 mRad to the ovaries despite gonadal shielding, the gonadal dose being 18 mRad (or higher in some studies) from a single exposure for a view of the kidneys, ureters and bladder (125).

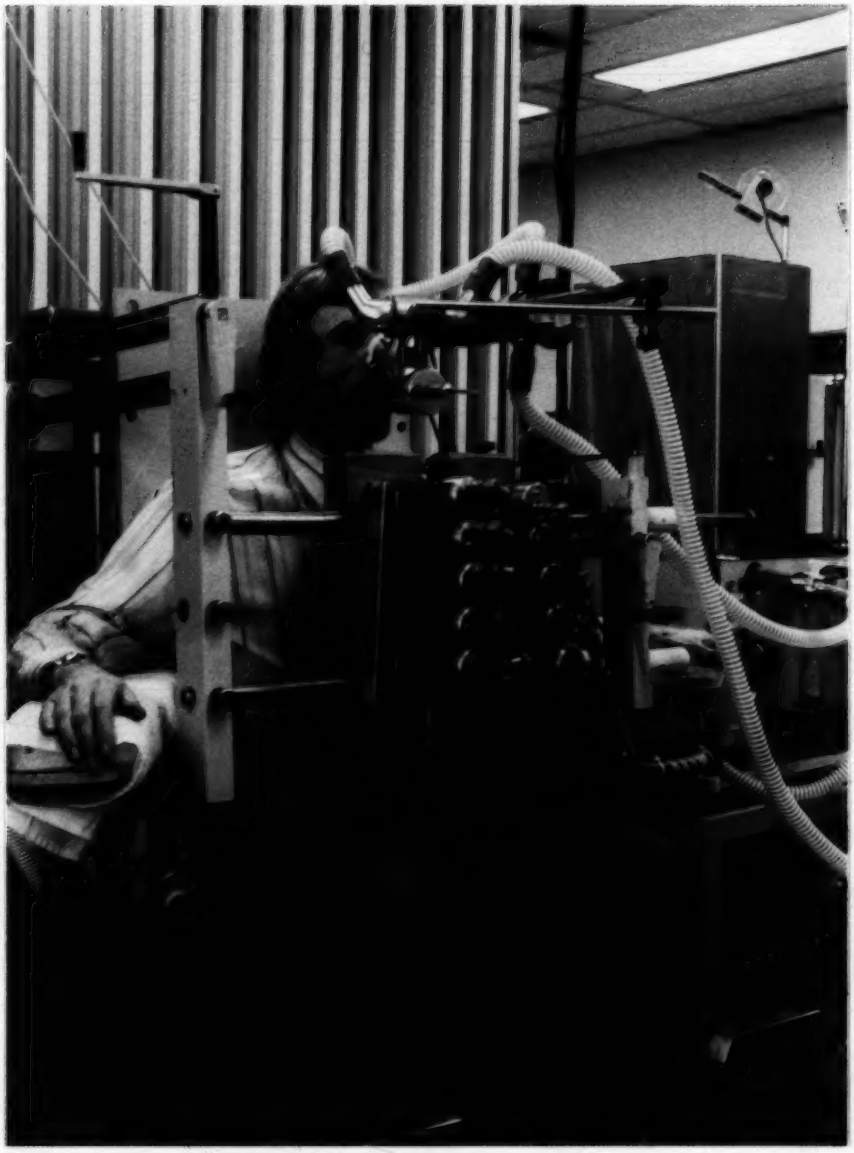


Plate 2 - The multiprobe detector system with a subject positioned correctly prior to testing.

d) Methods for measuring RVr/TLCr

Informed written consent is obtained (Appendix A). The subject sits between the anterior and posterior detectors with a seatbelt fastened around his hips to help maintain constant body position throughout subsequent testing (Plate 2). The height of the detectors is adjusted so that the centers of the upper detectors are approximately 5 cm below the lung apices. The AP separation of the detectors is now adjusted according to the patient size, ensuring adequate space for chest expansion to TLC. A pen mark placed on the supra-sternal notch helps center the patient and also aids repositioning of the subject for the subsequent ^{133}Xe studies. Both arms are positioned on arm rests to maintain constant body position, keeping the back and shoulders against the chair back.

Once the subject is correctly positioned he applies noseclips and breathes room air through a rubber mouthpiece and 3-way valve (deadspace 45 mls). For the next 30 seconds the background radioactivity count rate is recorded from all 10 detector pairs. At FRC the 3-way valve is turned, allowing the subject to breathe from a closed circuit spirometer containing 0.5 mCi ^{133}Xe per liter room air. Oxygen is added and carbon dioxide removed from the system to maintain constant volume and prevent hypoxia or hypercapnia. A couple of deep breaths are taken initially to speed equilibration of alveolar and spirometer ^{133}Xe gas concentrations (A, Fig. 20). The subject then continues

relaxed tidal breathing until equilibration has been achieved (B, Fig. 20). It takes approximately 3 minutes to achieve equilibration during which time the regional count rates from all detector pairs are plotted on a visual display unit as well as being stored in the computer memory.

Once equilibrated, the computer recording time base is changed to 0.5 seconds per frame. The subject inhales to TLC and holds his breath for 10 seconds with glottis open to allow a stable count rate to be recorded (C, Fig. 20). Next he is instructed to exhale to RV (D, Fig. 20). The spirometer tracing is watched carefully to see when RV is attained, at which point the mouthpiece 3-way value is opened to room air. A vital capacity breath of room air is inhaled and the subject again holds his breath at TLC for a further 10 seconds (E, Fig. 20). After this second breathhold he exhales to RV, the expired air being exhausted through a duct to a "radioactive" fume hood. Rapid washout of ^{133}Xe is now facilitated by hyperventilation into the exhaust system, 10% CO_2 (with balance O_2) being administered at the mouthpiece to prevent hypocapnia, thus helping the subject maintain hyperventilation for longer.

An initial rapid exponential decrease in regional count rates is seen as ^{133}Xe is cleared from the ventilated lung (E-F, Fig. 20) followed by a slower linear decline as absorbed ^{133}Xe is eliminated from the chest wall and lung parenchyma (F-G, Fig. 20). Data is collected for a total of 200 seconds, followed by a final 20 second background count.

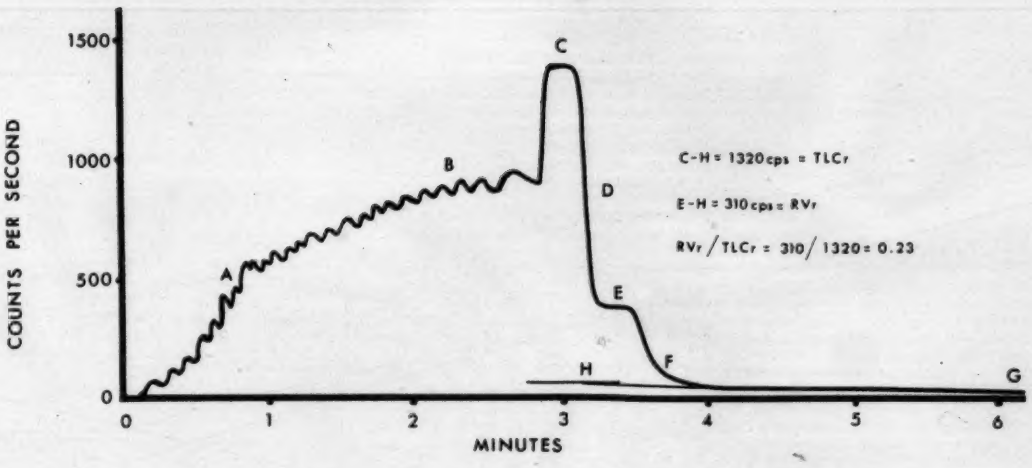


Fig. 20 - Typical curves obtained for washin and RVr/TLCr determination after rebreathing ^{133}Xe in air. For explanation see text.

e) Interpretation of data and calculation of RVr/TLCr

The mean count rate from each collinear detector pair is plotted on the Y axis against time on the X axis (Fig. 20).

For each of the 10 tracings obtained from the study the calculation of RVr/TLCr is performed. The linear portion of the washout curve is back-extrapolated to point H (Fig. 20) to correct for ^{133}Xe present in the chest wall and lung

parenchyma during the breathholds. The count rate at C (Fig. 20) is proportional to the TLCr volume. At point D the subject inhaled room air from RV back to TLC, thus the residual count rate at E is due to the ^{133}Xe gas still present in the lungs at RV. The lung volumes at which counts C and E are obtained are the same (i.e. TLC) and the counts may therefore be compared directly. The height E-H is therefore a measure of RVr volume and C-H measures TLCr volume. Thus the ratio of RVr/TLCr may be calculated for each of the 10 lung zones .

$$\text{i.e. } \frac{RVr}{TLCr} = \frac{EH}{CH}$$

(v) Methacholine Challenge Test

a) Introduction

As discussed in the literature review there are several methods of performing inhalational challenge tests, but these may basically be summarised as either intermittent or continuous aerosol generation techniques. The method described below is a simple, quick intermittent aerosol technique. The specification of the nebuliser determines the particle size and hence sites of deposition (see discussion in literature review (p.14), so this will be considered first.

b) Nebuliser specifications

The DeVilbiss D-45 (Fig. 21) is an all-plastic unit which has been developed as an advance on the glass

DeVilbiss No.40, the latter being the basis for much of the earlier studies on aerosol deposition. The DeVilbiss D-45 is very similar to the No.40, but, by using a ball baffle, a 50-60% increase in the output of useful aerosol is achieved without affecting the rate of atomisation (36). The aerosol droplets are 0.3-2 μm AMMD (DeVilbiss Inc., Personal communication) and it may be predicted that approximately 70% of the particles will be deposited within the respiratory system (including oropharynx) with 40-60% reaching the alveoli (43,126).

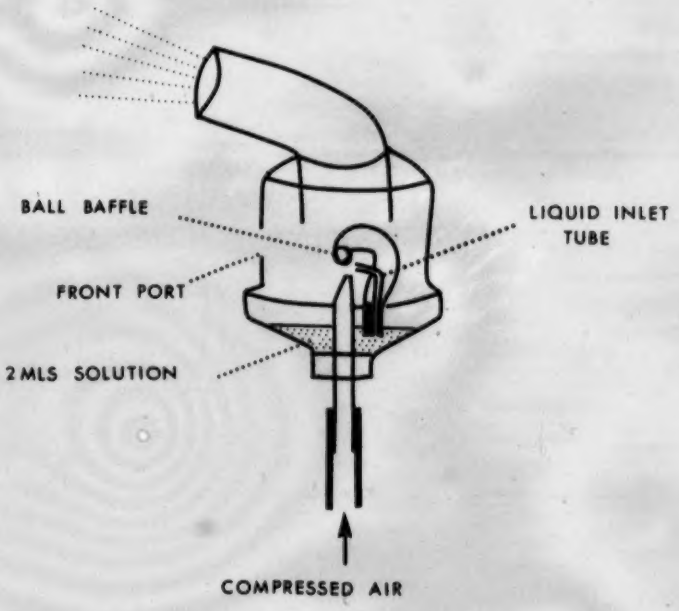


Fig. 21 - The DeVilbiss D-45 plastic nebuliser.

c) Preparation of methacholine chloride solutions

Six different concentrations of methacholine chloride solution (0.5, 1.0, 2.0, 5.0, 10.0, 25.0 mg/ml) are prepared under sterile conditions in the University of Alberta Hospital Pharmacy. Saline and phenol diluent is used for optimum stability and sterility, the addition of a small amount of sodium bicarbonate achieving a neutral pH. An aqueous solution is unsuitable since even in a refrigerator this deteriorates, particularly if in alkaline solution, the Merck Index (127) suggesting aqueous solution be discarded after no longer than two weeks. MacDonald (128) showed that methacholine dissolved in saline loses 10% potency in 48 days if stored at room temperature, but a similar deterioration took 128 days if kept at 4°C. The diluent chosen contains 0.5% NaCl, 0.275% $NaHCO_3$ and 0.4% phenol, achieving a pH of 7.0 to minimise chemical trauma to the airways (35).

d) Performance of challenge test

To each of 6 nebulisers, 2 ml of a methacholine solution is added and the nebuliser appropriately labelled. Two ml of the diluent is added to a seventh nebuliser to provide a baseline control. The control nebuliser is taken and connected, with the front port open, to a timed dosimeter. This, in turn, is connected via a Melco flowmeter set at 10 ℓ/minute to a pressurised air tank, the outlet pressure reduced to 20 psi (Fig. 22). The

dosimeter fires 12 times per minute, allowing 5 seconds for each breath cycle. At 0.5 seconds before firing a red light illuminates on the dosimeter; the dosimeter then delivers a constant volume and flow rate of aerosol for 0.5 seconds after activation, the cycle then repeating.

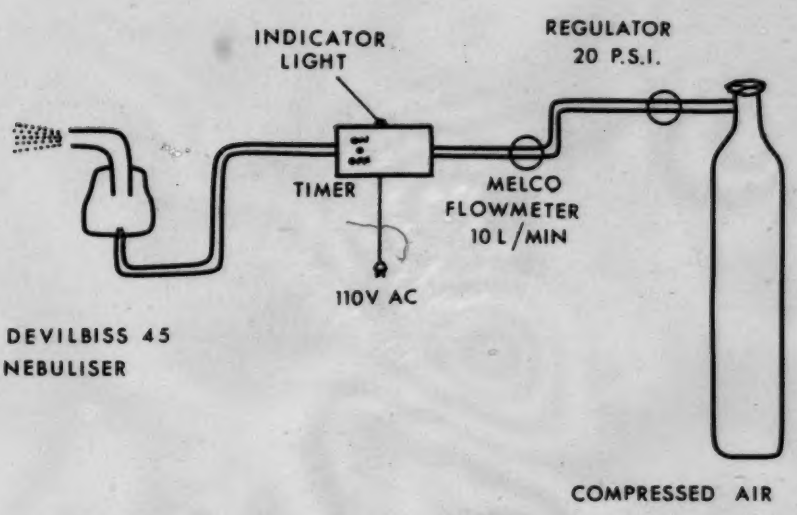


Fig. 22 - Arrangement of equipment for inhalational challenge testing.

The subject performs a flow-volume loop manoeuver, recorded on a graphic plotter (Cavitron Inc SC-20A spirometric analyser) from which FVC, FEV_1, $\dot{V}_{50}$ and $\dot{V}_{75}$ are calculated.

The height of the nebuliser is adjusted for the subject's comfort whilst seated and he is asked to continue

tidal breathing through his mouth, his lips loosely around the nebuliser mouthpiece. The dosimeter is now activated and the subject instructed to inhale deeply and slowly from FRC as soon as the red light appears on the dosimeter. A metered dose of aerosol is therefore delivered 0.5 seconds after the start of inhalation, for a total period of 0.5 seconds. After a full inhalation he holds his breath for 2 seconds, then exhales to FRC ready for the next breath of aerosol. Five metered inhalations of the diluent (control solution) are administered in this manner. At 1 minute and 4 minutes after finishing the 5 inhalations, flow-volume loops are recorded to determine FVC, FEV_1, $\dot{V}_{50}$ and $\dot{V}_{75}$.

If there is no sensitivity to the diluent (see below), the sequence is repeated with 0.5 mg/ml methacholine solution and subsequently with sequentially increasing concentrations. Five minutes is allowed between each different test solution. The criteria for test termination are given below.

e) Criteria for challenge test termination

After the control (diluent) solution has been administered, a 5.2% (2σ from normal) fall in FEV_1 from the initial pre-challenge level, or a deterioration in lung function on the flow-volume loop, indicates hypersensitivity to the diluent (phenol/saline/bicarbonate). The test must then be terminated (35,54).

If no deterioration occurs after the diluent, then the

test continues until a sustained 20% fall in FEV_1 from the post-diluent value is observed. Whenever a fall in FEV_1 is observed on the 1 minute post-inhalational spirometry, particular note is paid to the values at 4 minutes. If, by 4 minutes, recovery has occurred, then the test proceeds to the next concentration. If the fall in FEV_1 is sustained, with FEV_1 20% less than control, the test is terminated. The patient then proceeds to the second measurement of RVr/TLCr to determine the pattern of alteration in regional lung function induced by methacholine inhalation.

If no significant fall in FEV_1 is observed, the test is terminated after the 4 minute flow-volume loop following inhalation of the 25 mg/ml methacholine solution. The subject then has RVr/TLCr measurements repeated.

f) Recording the challenge test results

Both the concentration of methacholine (mg/ml) and the cumulative dose units administered are recorded with the corresponding spirometric data (FVC, FEV_1, $\dot{V}_{50}$, $\dot{V}_{75}$) at 1 and 4 minutes following each set of inhalations. The cumulative dose is calculated having defined 1 dose unit as being equal to 1 metered breath of 1 mg/ml methacholine solution (35). Clearly the exact dose is specific to the dosimeter and nebuliser used, allowing comparison of results from challenge testing performed on different subjects in our laboratory. The concentration resulting in a 20% fall in FEV_1 (PC_{20FEV_1}) is recorded if the challenge test is

positive, this being a measure of the airways sensitivity to methacholine (129,130). The slope of the dose-response curve reflects the airways reactivity to methacholine (Fig. 23). Thus the slope of the curve is steep with highly reactive airways and more gradual with lower degrees of reactivity.

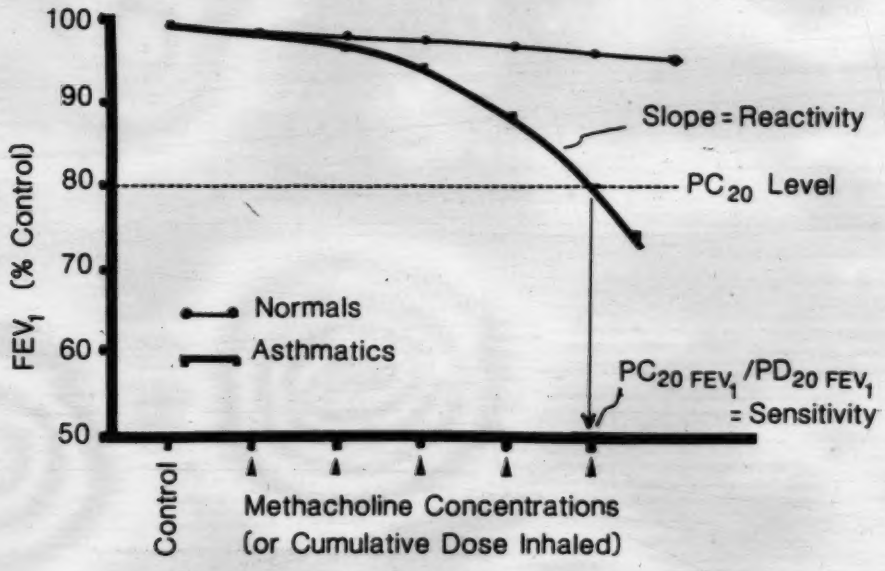


Fig. 23 - Dose-response curve for inhaled methacholine. The difference between airways sensitivity (PC_{20FEV_1}) and reactivity are illustrated comparing a normal subject with an asthmatic.

Isovolume $\dot{V}_{50}$ and $\dot{V}_{75}$ are determined and recorded with the other spirometric data in tabular form, although the results are distributed to the referring physician as a graph for ease of interpretation (Fig. 24).

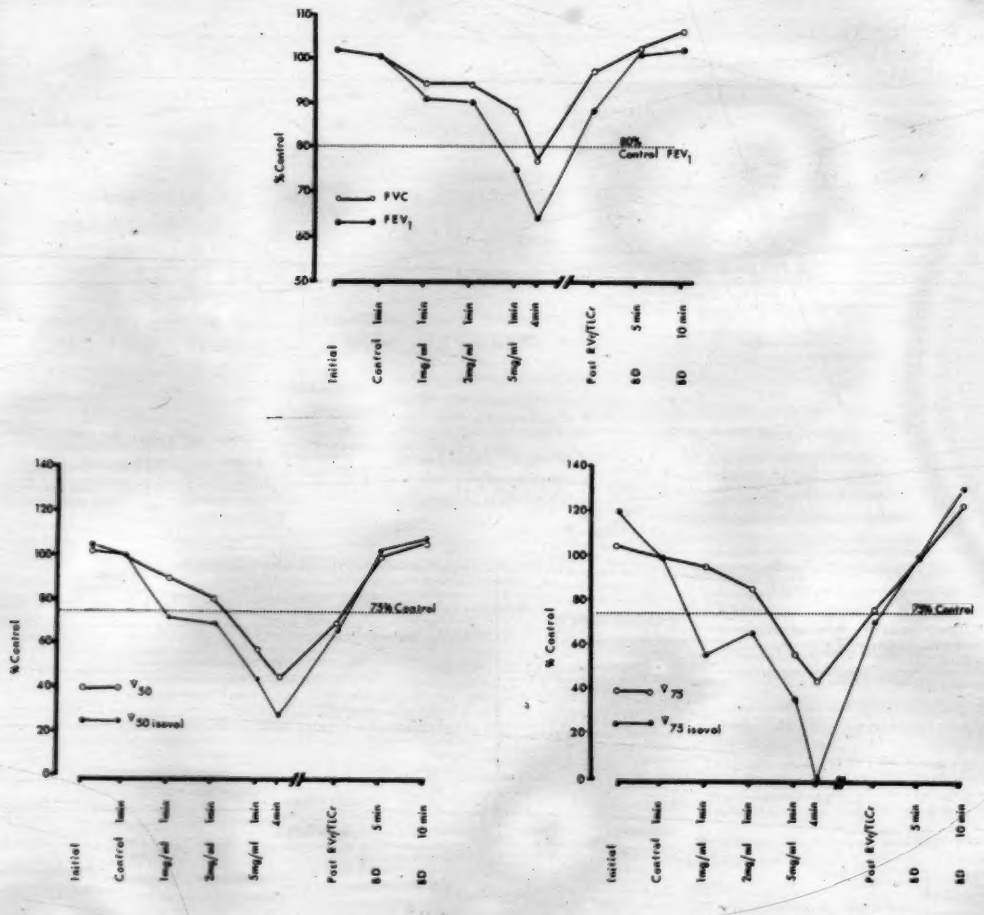


Fig. 24 - The effect of methacholine inhalation on lung function (Subject 5). The upper graph shows changes in FVC and FEV_1. Changes in $\dot{V}_{50}$ and $\dot{V}_{50isovol}$ are shown in the bottom left graph and $\dot{V}_{75}$ and $\dot{V}_{75isovol}$ in the bottom right graph. The response to bronchodilator (BD; Salbutamol) is also illustrated.

g) Determination of effect of methacholine on regional lung function

The RVr/TLCr measurements are repeated using the same technique as previously described. As no other patients have been tested in the interval after the first determination of RVr/TLCr, the repositioning of the patient in the apparatus has proved easy. The absolute and percentage change in RVr/TLCr between the pre-challenge and post-challenge measurements are determined.

h) Bronchodilator administration and final RVr/TLCr measurement

After completing the second RVr/TLCr measurements, the flow-volume loop is repeated and 2 puffs of Salbutamol administered from a standard pressurised inhaler. The flow-volume loop is repeated again 5 and 10 minutes after administration of Salbutamol. If complete reversal does not occur, a further 1 or 2 puffs of Salbutamol are given. Once complete reversal of effects caused by the methacholine has been comfirmed, a third determination of RVr/TLCr is performed to assess reversal of regional lung function changes.

(i) Potential risks and precautions

The relative risks of the radiation have been mentioned already. No pregnant females are studied with radiospirometry, even though the actual dose to the gonads

is small. Dyspnea, wheezing and chest tightness may result from inhalation of methacholine in sensitive subjects. All symptoms and spirometric changes are generally readily reversed by 2 puffs of bronchodilator, but facilities for administering Salbutamol solution by sidestream nebulisation must always be available during challenge testing in case of a severe reaction. Intravenous aminophylline and oxygen are also kept in the room where challenge testing is performed. Written consent is obtained from all subjects before commencing testing (see Appendix A) after complete explanation of the procedures involved.

Chapter IV

RESULTS

Twenty six subjects with mean age 34 years were studied between September 1982 and January 1983. Fifteen were specifically referred for bronchial provocation testing by physicians from the Pulmonary Division, University of Alberta Hospital. The presenting symptoms of these patients were exertional dyspnea (eleven), episodic wheezing (six), persistent nonproductive cough (six) and chest "tightness" in association with breathlessness (four). Most had more than one symptom as shown in Table 9 (Appendix B). All were nonsmokers and there was no clinical, radiographic or hematological evidence to suggest chronic lung disease. Eleven healthy volunteers were also studied, only one of whom gave a history to suggest atopy (subsequently confirmed) and none of whom had any respiratory symptomatology.

The anthropomorphic data of all subjects is given in Table 9 (Appendix B). An indication of atopy is also given in this table. This was assessed by means of clinical history and skin scratch testing using 18 common allergens (Hollister-Stier, Division of Cutter Labs Inc., USA) in 23 of the 26 subjects. Seven of the subjects had moderately to markedly positive scratch tests, with lesser degrees of sensitivity in 5 subjects and 11 were negative for the allergens used.

In all the tables of results subjects have been grouped according to the degree of airways responsiveness to inhaled methacholine. "Unequivocal responders" (subjects 1-12)

showed a 20% or greater fall in FEV_1 from control values whilst "partial responders" (subjects 13-18) had a change in RVr/TLCr ratios associated with decreases in $\dot{V}_{50}$, $\dot{V}_{75}$ and isovolume flow rates, although without a significant sustained fall in FEV_1. Subjects 19-26 showed no response to methacholine inhalation ("Nonresponders"). This will be further explained later in this chapter with presentation of the actual challenge test and RVr/TLCr results. Only one of the referred patients (subject 24) showed no response to methacholine inhalation. Two volunteers (subjects 5 and 7) were "unequivocal responders" and two (subjects 15 and 17) showed partial responsiveness to inhaled methacholine. Table 3 summarises the anthropomorphic data for the different subject groups.

The pulmonary function test results of all subjects are given in Table 10 (Appendix C). Normal values used by the University of Alberta Pulmonary Function Laboratory have been derived from the published literature (131-136). The percent of predicted values are recorded for each measurement. The FEV_1, however, is expressed as percent of VC (normal > 75%) and ET-FEV_1 as percent of ERV (normal >75%). Normal values for closing volume and closing capacity were taken from the data of Buist and Ross (81), with range for the slope of phase III of the single breath N_2 washout given by Anthonisen et al (78).

TABLE 3

Summary of Anthropomorphic Data

(mean ± 1SD)

	Age yrs	#Female	Height cm	Weight kg	#Atopic
All subjects (n=26)	34 ± 11	15	170 ± 9	68.5 ± 9.6	13/23
Nonresponders (n=8)	30 ± 6	4	176 ± 6	68.2 ± 8.5	2/7
All responders (n=18)	35 ± 12	11	168 ± 9	68.7 ± 10.3	11/16
Unequivocal responders (n=12)	34 ± 15	8	166 ± 8	68.0 ± 11.0	8/11
Partial responders (n=6)	36 ± 5	3	170 ± 11	70.1 ± 9.6	2/5

Ninety-five percent of normal subjects have been shown to fall within the range of the predicted mean value for each lung function parameter ± 1.645 times the standard error of the estimate (SEE). The results for end-expiratory flow rates from subject 2,14,15 and 25 were outside this range, as was the closing capacity measurement for subject 14. The mean data for the different subject groups is summarised in Table 11 (Appendix C). The percentage values and standard deviations are also given to indicate the approximation to the predicted mean in each group. No statistically significant difference in pulmonary function was shown between responders and nonresponsders by unpaired Student's t-test analysis of the data.

Table 12 (Appendix D) shows the response to inhaled methacholine in each subject with the mean results for the subgroups given in Table 13 (Appendix D). The actual control (post diluent) values are given with subsequent results expressed as a percentage of the control value. The post-diluent (control) values did not differ by more than 5.2% (2 σ) from baseline values recorded immediately prior to commencing the challenge testing, even in the subjects subsequently shown to have the highest levels of airways reactivity. The post-methacholine values recorded indicate the spirometric results obtained 4 minutes after the final dose of methacholine had been administered. These therefore represent the results at the termination of the test, either after a 20% fall in FEV_1 had occurred or the 25 mg/ml

solution of methacholine had been administered. The PC_{20FEV_1} (in mg/ml) and cumulative dose administered, (where 1 dose unit equals 1 metered breath of 1 mg/ml methacholine solution) are indicated.

Only one subject (number 1) reacted to the most dilute (0.5 mg/ml) methacholine solution, the mean PC_{20FEV_1} for unequivocal responders being 8.7 ± 8.2 mg/ml (mean ± 1SD). Six subjects (subjects 13-18) did not show a sustained fall in FEV_1 below 80% of control, but did demonstrate a 25% or greater fall in $\dot{V}_{50}$, $\dot{V}_{75}$ or isovolume flow rates after inhaling the 25 mg/ml methacholine solution. Eight subjects (subjects 19-26) had neither a significant fall in FEV_1 nor end-expiratory flow rates, although reduction in $\dot{V}_{75isovol}$ was observed in 5 of these subjects.

The persistence of bronchospasm throughout the second assessment of RVr/TLCr was confirmed by repeating spirometry immediately after completion of the xenon study, prior to administration of bronchodilator. Whilst there was a tendency for the effect of the methacholine to wear off, the mean FEV_1 (c.f. mean values in Table 13, Appendix D) for the "unequivocal responders" remained significantly decreased at 81 ± 10% of control ($\bar{x}$ ± 1SD), only one subject in this group (Subject 8) having an FEV_1 above 90% of control. The mean $\dot{V}_{50}$ and $\dot{V}_{75}$ for the "unequivocal responders" were 63 ± 17% control and 66 ± 17% control respectively prior to bronchodilator administration. "Partial responders" had a mean FEV_1 of 95 ± 6% control, $\dot{V}_{50}$ 86 ± 4% control and

$\dot{V}_{75}$ 81 ± 11% control immediately after the completion of the RVr/TLCr measurement. Corresponding mean values for the group of nonresponders were FEV_1 96 ± 3% control, $\dot{V}_{50}$ 91 ± 6% control and $\dot{V}_{75}$ 87 ± 14% control.

Post-bronchodilator results shown in Table 12 (Appendix D) were recorded 10 minutes after administration of 2 puffs of Salbutamol (Ventolin) from a standard pressurised cannister inhaler, prior to the third measurement of RVr/TLCr.

The lung zones referred to in Table 14 (Appendix E) follow the convention outlined in Chapter III (page 72). All subjects had initial (pre-challenge) RVr/TLCr measurements within the normal range for their respective age groups as derived by Jones et al (104). No significant difference in baseline pre-challenge RVr/TLCr results between responders and nonresponders, nor between the "unequivocal" and "partial" responders, was shown by unpaired Student's t-test analysis. The relationship of the overall mean (all subjects) RVr/TLCr measurements to the previously published normal values is shown in Figure 25.

The RVr/TLCr results of all subjects for the initial, post-methacholine and post-bronchodilator studies are given in Table 14 (Appendix E). A summary of the mean RVr/TLCr values for each of the subgroupings is given in Table 15 (Appendix E). Following methacholine inhalation no significant change in RVr/TLCr ratios were demonstrated in subjects 19-26, with no change either after subsequent

bronchodilator inhalation.

Subjects 1-18 showed a patchy pattern of change in RVr/TLCr results following methacholine inhalation, greatest changes being observed in subjects 1-12 ("unequivocal responders"). In subject 12 the lower and mid zones of both lungs were affected only slightly and the RVr/TLCr ratio of zone 8 (left mid zone) did not change at all following methacholine. Subjects 9 and 11 showed maximum changes at the lung bases. In contrast a more generalised change was observed in subject 10. Figures 26-29 illustrate these different patterns of change in RVr/TLCr following methacholine inhalation, also illustrating the good correlation of RVr/TLCr values for corresponding zones of the two lungs prior to challenge testing.

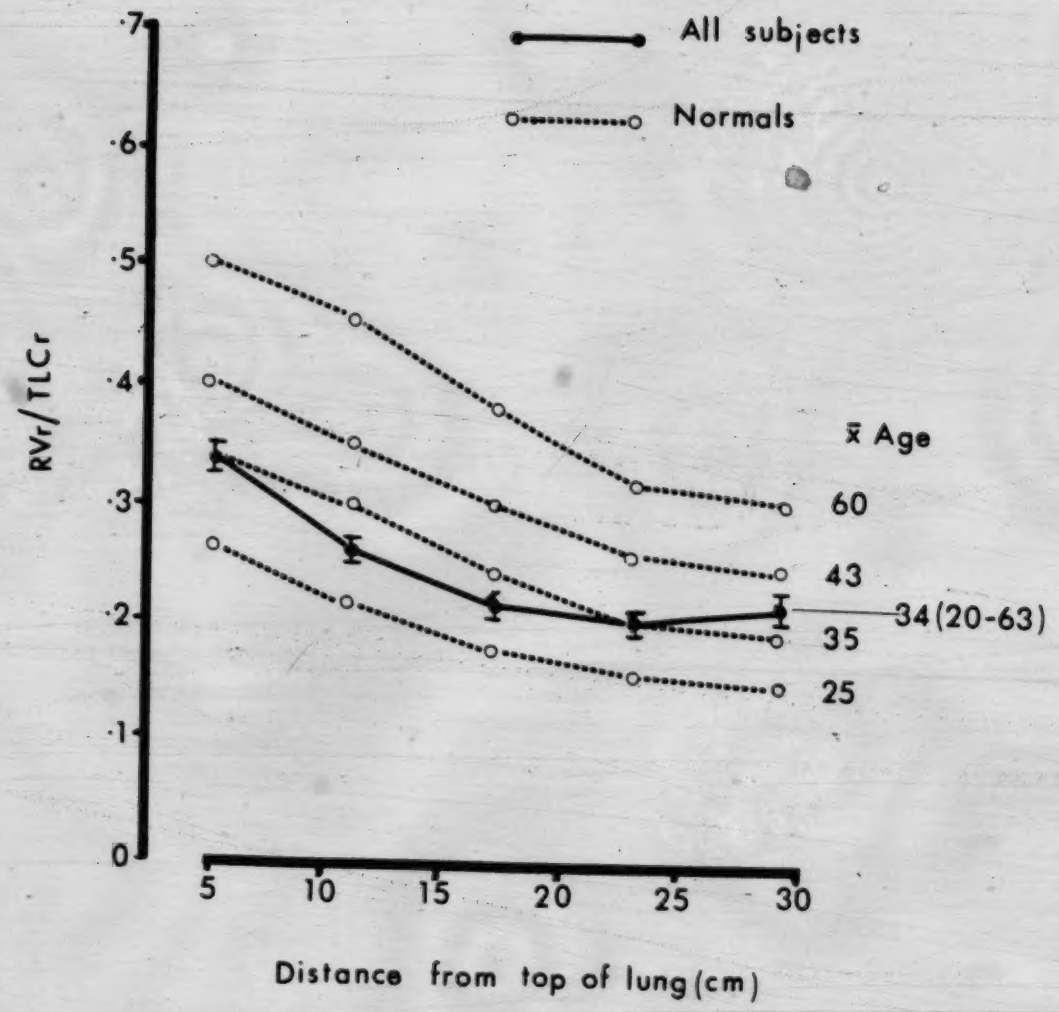


Fig. 25 - Mean RVr/TLCr ± 1SEM prior to challenge testing of all subjects compared to published normal data (104). The mean age of each group is indicated next to the value for the lower lung region. The age range of the study group is given in parentheses.

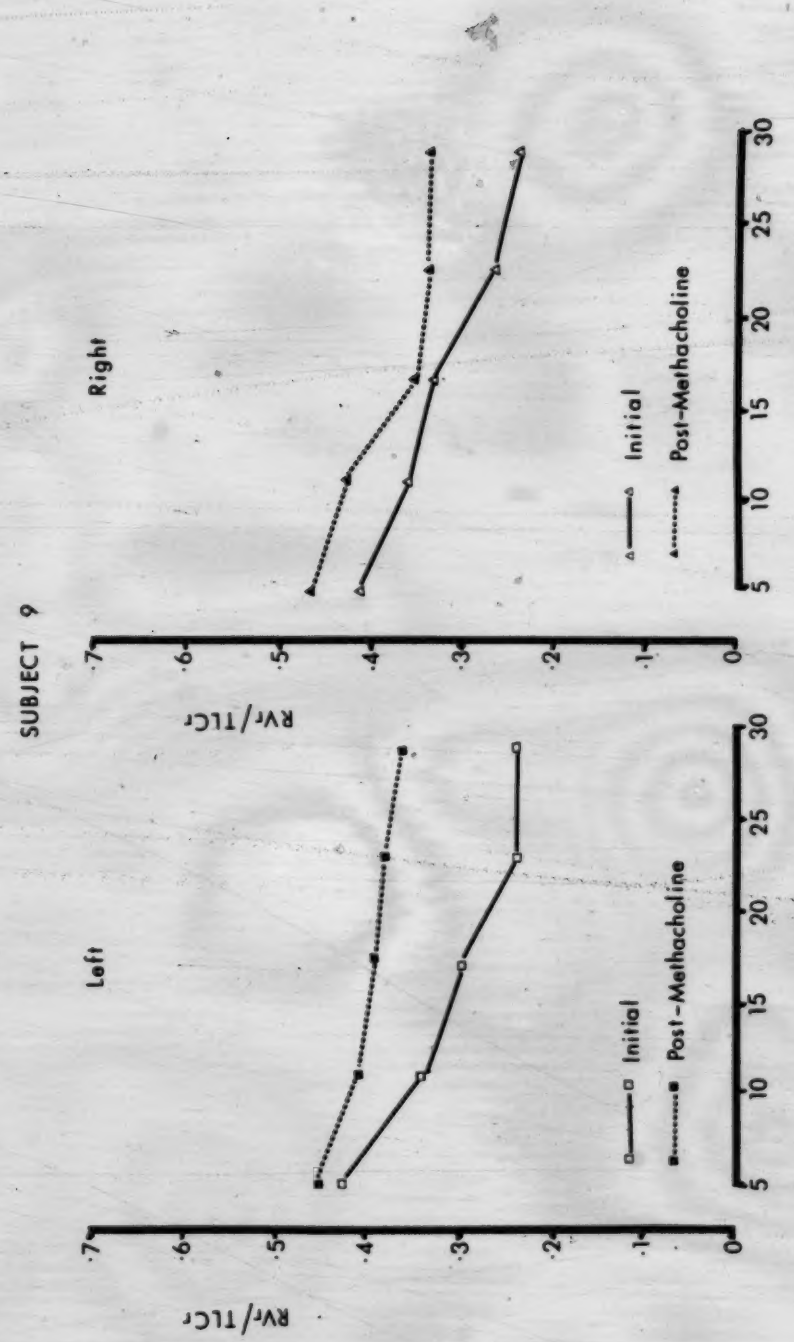


Fig. 26 - The patchy effect of methacholine inhalation on RVr/TLCr ratios of left and right lungs in subject 9.

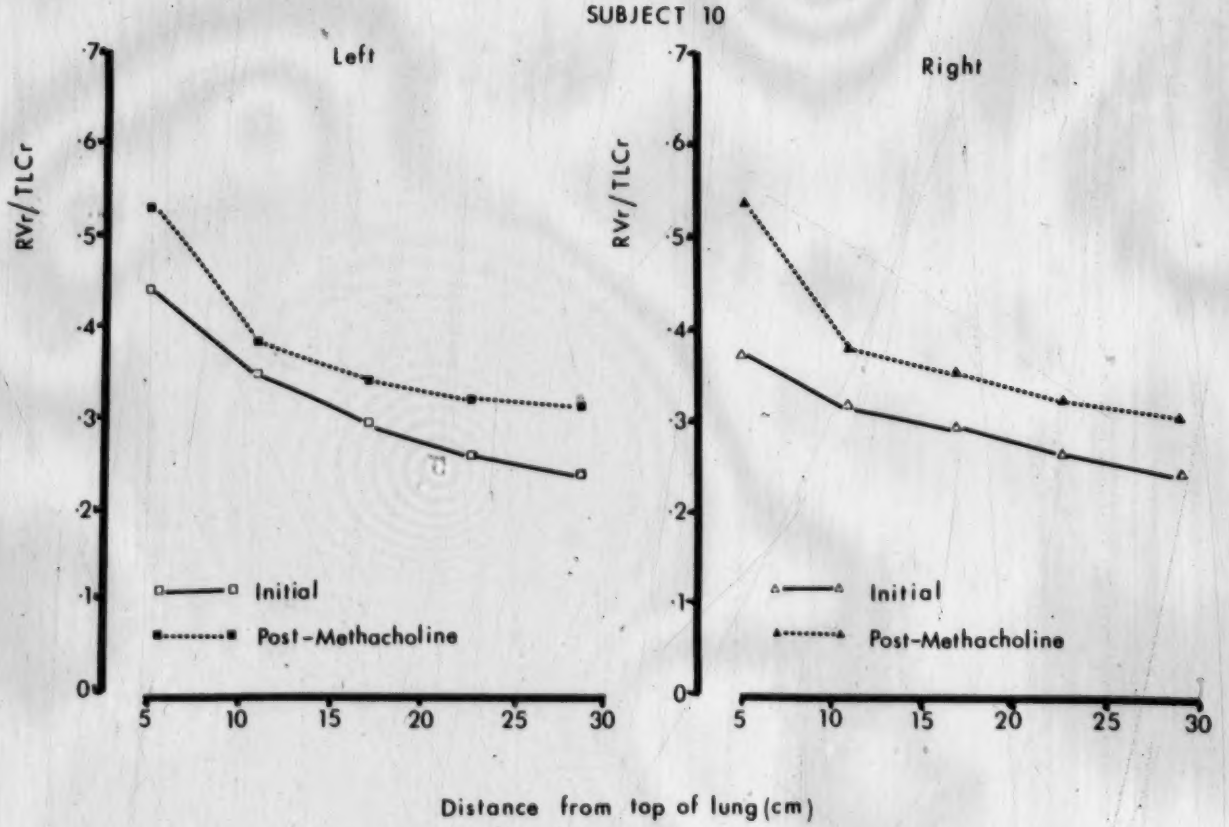


Fig. 27 - The effect of methacholine inhalation on RVr/TLCr ratios of both lungs in subject 10.

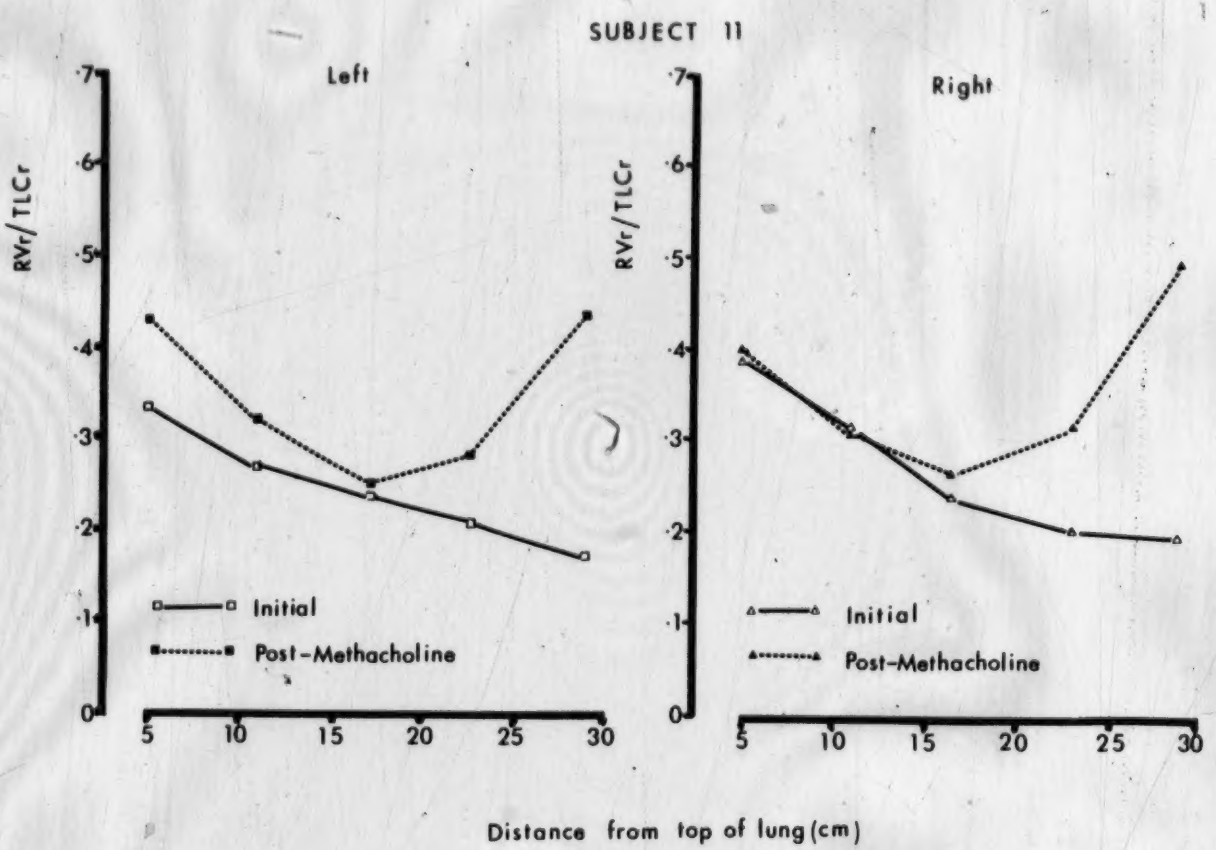


Fig. 28 - The effect of methacholine inhalation on RVr/TLCr ratios of both lungs in subject 11.

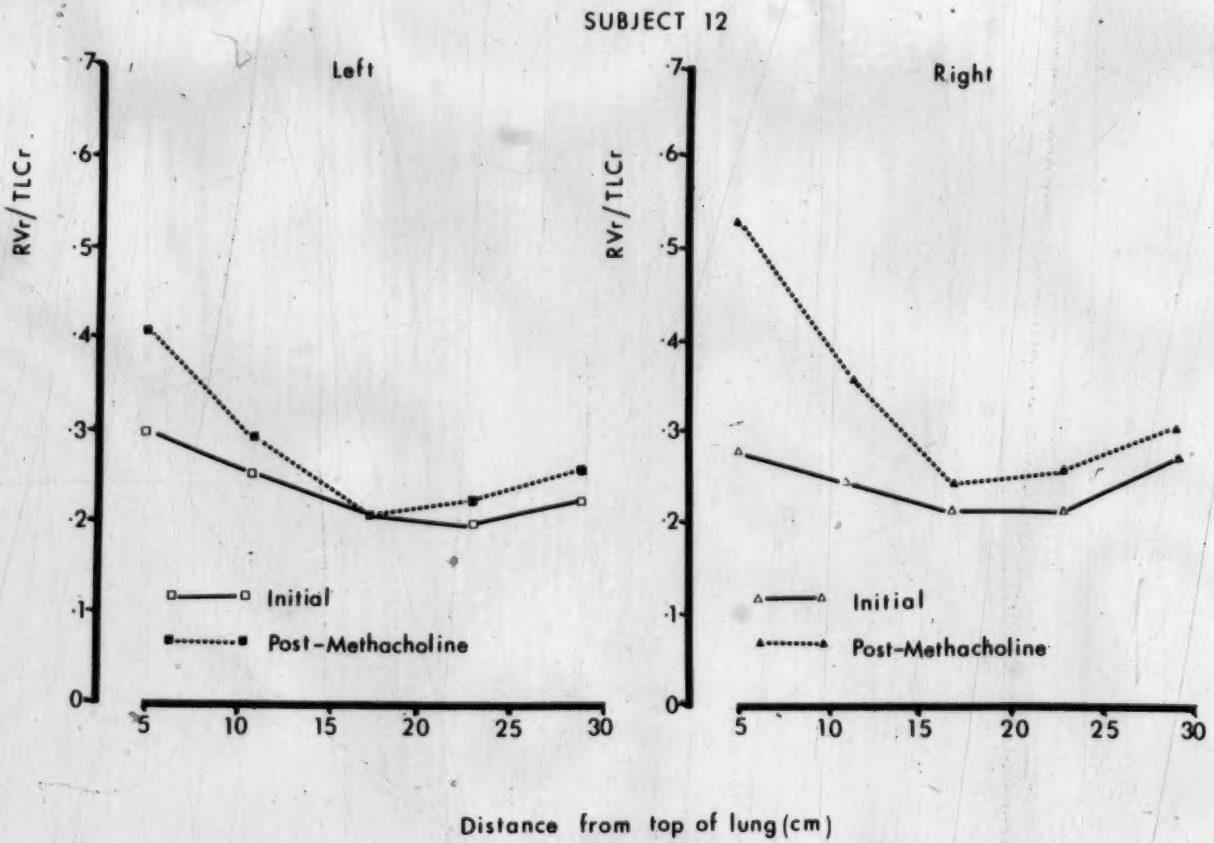


Fig. 29 - The effect of methacholine inhalation on RVr/TLCr ratios of both lungs in subject 12.

The pre and post methacholine results of all subjects were analysed by paired Student's t-tests. Significant p values were obtained for the responders (subjects 1-18) at $p<0.01$ in all zones except zone 6 (left apex) where the p value was 0.02 (Table 16, Appendix E). Analysing the "unequivocal responders" independently, the changes in mean RVr/TLCr values were seen to be significant at $p<0.001$ in zones 3,4,5,9 and 10 (i.e. lower and mid zones), the six partial responders (13-18) having smaller mean changes in RVr/TLCr ratios.

Following inhalation of bronchodilator there was improvement in RVr/TLCr ratios, although in some subjects the values did not return to the baseline levels in the upper zones despite normalization of spirometric data (Table 14, Appendix D). In subject 3, for example, the RVr/TLCr in zone 1 was 0.19 initially, 0.37 post-methacholine and 0.30 post-bronchodilator. Greatest improvement was observed at the lung bases, with the highest degrees of statistical significance when the post-challenge and post-bronchodilator values were compared in the "unequivocal responders" for these regions. The post-methacholine and post-bronchodilator RVr/TLCr ratios were not significantly different in the partial responders (Tables 15 and 16, Appendix E). No significant difference existed between the initial (pre-challenge) and post-bronchodilator RVr/TLCr results for any of the subject subgroupings despite the incomplete responses to bronchodilator observed.

The absolute (numerical) differences between the upper (zones 1 and 6) and lower (zones 5 and 10) RVr/TLCr measurements for the "unequivocal responders" increased from 0.14 ± .05 ($\bar{x}$ ± 1SD) initially to 0.17 ± .14 post-methacholine and 0.19 ± .11 post-bronchodilator. "Partial responders" had an initial difference of 0.16 ± .05, 0.14 ± .06 post-methacholine and 0.16 ± .07 post-bronchodilator. The corresponding values for nonresponders were 0.14 ± .05 initially, 0.13 ± .04 post-metacholine and 0.13 ± .04 post-bronchodilator. These slight changes in the absolute differences between upper and lower zone RVr/TLCr values were not statistically significant for any of the subject groups.

The correlation coefficient between pre-challenge RVr/TLCr values obtained from corresponding regions of the left and right lung was 0.94 (Fig.30). The data was therefore reduced further by analysing the results from corresponding zones in the two lungs together. The "n" values in the subsequent tables are therefore twice those in Table 15 (Appendix E). Figure 25 shows the relationship of the overall mean pre-challenge RVr/TLCr results to the normal values of Jones et al (104). Table 4 shows the mean results from responders and nonresponders obtained from the 3 studies, the lung zones now described in terms of distance from the top of the lungs. The consistency of the RVr/TLCr results obtained in the 3 studies from subjects 19-26

(nonresponders) is clearly demonstrated, as well as the identical mean RVr/TLCr values for responders and nonresponders prior to challenge testing.

In Table 5 the results obtained from the 12 "unequivocal responders" and 6 "partial responders" are contrasted. The greatest percentage increases in RVr/TLCr values were observed in the "unequivocal responders", being a 50% increase 23 cm from the lung apex and a 45% increase 29 cm from the lung apex. The "partial responders" showed an 18-20% increase in RVr/TLCr in all lung zones, although still greater than the minor variations observed in the nonresponders (Table 6).

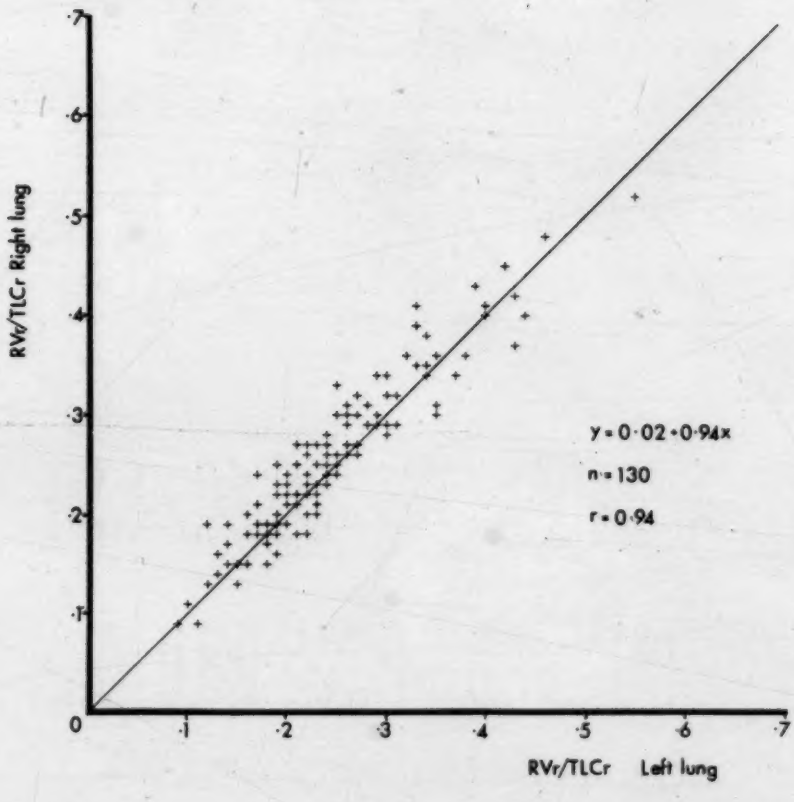


Fig. 30 - Correlation between pre-challenge RVr/TLCr values for all corresponding regions of both lungs obtained for all subjects. The line of identity is shown.

TABLE 4

Mean RVr/TLCr ± 1SD for responders and nonresponders pre- and post-methacholine and post-bronchodilator

	Distance from top of lung				
	5cm	11cm	17cm	23cm	29cm
Responders (n=36)					
Initial	.34 ± .10	.26 ± .07	.22 ± .06	.20 ± .04	.21 ± .05
Post-methacholine	.45 ± .13	.35 ± .10	.30 ± .07	.28 ± .06	.29 ± .07
Post-bronchodilator	.38 ± .12	.29 ± .08	.24 ± .05	.21 ± .05	.21 ± .04
Nonresponders (n=16)					
Initial	.34 ± .06	.26 ± .05	.22 ± .04	.21 ± .04	.19 ± .05
Post-methacholine	.33 ± .06	.26 ± .05	.22 ± .05	.20 ± .04	.20 ± .05
Post-bronchodilator	.32 ± .08	.26 ± .07	.23 ± .07	.21 ± .05	.19 ± .05
All subjects (n=52)					
Initial	.34 ± .09	.26 ± .07	.22 ± .05	.20 ± .04	.21 ± .05

TABLE 5

Mean RVr/TLCr ± 1SD for "unequivocal responders" and "partial responders" pre- and post-methacholine and post-bronchodilator

	Distance from top of lung				
	5cm	11cm	17cm	23cm	29cm
Unequivocal Responders (n=24)					
Initial	.34 ± .11	.27 ± .08	.23 ± .06	.20 ± .05	.22 ± .05
Post-methacholine	.47 ± .15	.38 ± .10	.32 ± .06	.30 ± .04	.32 ± .06
Post-bronchodilator	.40 ± .13	.30 ± .08	.24 ± .06	.21 ± .05	.21 ± .05
Partial Responders (n=12)					
Initial	.34 ± .07	.25 ± .05	.22 ± .04	.20 ± .04	.21 ± .07
Post-methacholine	.40 ± .07	.30 ± .06	.26 ± .06	.24 ± .06	.25 ± .06
Post-bronchodilator	.35 ± .07	.28 ± .06	.23 ± .05	.21 ± .05	.20 ± .03

TABLE 6

Percentage increase in RVr/TLCr following methacholine inhalation

	Distance from top of lung				
	5cm	11cm	17cm	23cm	29cm
All Responders	32	35	36	40	38
Unequivocal Responders	38	41	39	50	45
Partial Responders	18	20	18	20	19
Nonresponders	-3	0	0	-5	+5

When analysed by paired Student's t-tests the highest degree of significance was observed in the regions 17,23 and 29 cm from the lung apices, both for all responders and "unequivocal responders". The post-methacholine and post-bronchodilator results did not differ significantly at the lung apices when the "unequivocal responders" were analysed separately from all responders ($p>0.05$). "Partial responders" showed a statistically significant change in RVr/TLCr ratios at the 0.05 level only in the regions 11 cm from the tops of the lungs. The post-methacholine and post-bronchodilator values differed significantly ($p<0.05$) only at the lung base for "partial responders". Pre-challenge and post-bronchodilator RVr/TLCr results were not significantly different in any subgroups of subjects. Table 7 shows the results of paired t-test analysis of the data.

TABLE 7

Probability values resulting from paired t-testing for RVr/TLCr comparisons indicate

(NS = not significant at 0.05 level)

	Distance from top of lung				
	5cm	11cm	17cm	23cm	29cm
All Responders					
Initial vs Meth.	<.001	<.0001	<.0001	<.0001	<.0001
Meth. vs BD	<.05	<.01	<.001	<.0001	<.0001
Initial vs BD	NS	NS	NS	NS	NS
Unequivocal Responders					
Initial vs Meth.	<.01	<.001	<.0001	<.0001	<.0001
Meth.vs BD	NS	<.01	<.0001	<.0001	<.0001
Initial vs BD	NS	NS	NS	NS	NS
Partial Responders					
Initial vs Meth.	NS	<.05	NS	NS	NS
Meth.vs BD	NS	NS	NS	NS	<.05
Initial vs BD	NS	NS	NS	NS	NS
Nonresponders					
Initial vs Meth.	NS	NS	NS	NS	NS
Meth.vs BD	NS	NS	NS	NS	NS
Initial vs BD	NS	NS	NS	NS	NS

Meth = Post-methacholine BD = Post-bronchodilator

The mean ratios of upper (regions 1 and 6) to lower (regions 5 and 10) zones RVr/TLCr (U/L ratio) were 1.70 ± .46 for all subjects, 1.79 ± .39 for the non-responders and 1.65 ± .48 for responders (mean ± 1SD). Following methacholine inhalation these ratios fell slightly in all groups. After inhaling bronchodilator the upper to lower zone ratios increased to above the pre challenge values in all responders ("partial" as well as "unequivocal").

These changes (Table 8) were not significant at the 0.05 level for "partial responders" or nonresponders. The decrease in U/L ratio post-methacholine was significant at the 0.05 level for all responders but not for "unequivocal responders". The post-methacholine U/L ratios differed significantly ($p<0.001$) from the U/L ratios post-bronchodilator for all responders as well as for "unequivocal responders". The increased post-bronchodilator U/L ratio comparing with the initial ratio was significant ($p<0.05$) for "unequivocal responders" only. These results are presented in Table 8 and illustrated in Figures 31 and 32.

The numerical difference in RVr/TLCr values between the upper (zones 1 and 6) and lower (zones 5 and 10) regions did not change significantly ($p>0.05$) in any of the subject groupings between the three measurements recorded.

Figures 33-36 present the data from Tables 6-9 graphically. The X axis and the left hand Y axis show the

distance from the top of the lung and the RVr/TLCr ratio in all graphs. The position of the right hand Y axis, showing the percentage increase in RVr/TLCr following methacholine inhalation, varies between graphs in order to maintain clarity.

TABLE 8

U/L ratio for all subject groups (Mean ± 1SD)

	Initial	Meth	BD
All Subjects (n=52)	1.70 ± .46		
Nonresponders (n=16)	1.79 ± .39	1.65 ± .26	1.72 ± .34
All Responders (n=36)	1.65 ± .48	1.55 ± .47	1.85 ± .51
Unequivocal Responders (n=24)	1.60 ± .50	1.50 ± .49	1.90 ± .53
Partial Responders (n=12)	1.72 ± .39	1.60 ± .41	1.75 ± .51

Meth = Post-methacholine BD = Post-bronchodilator

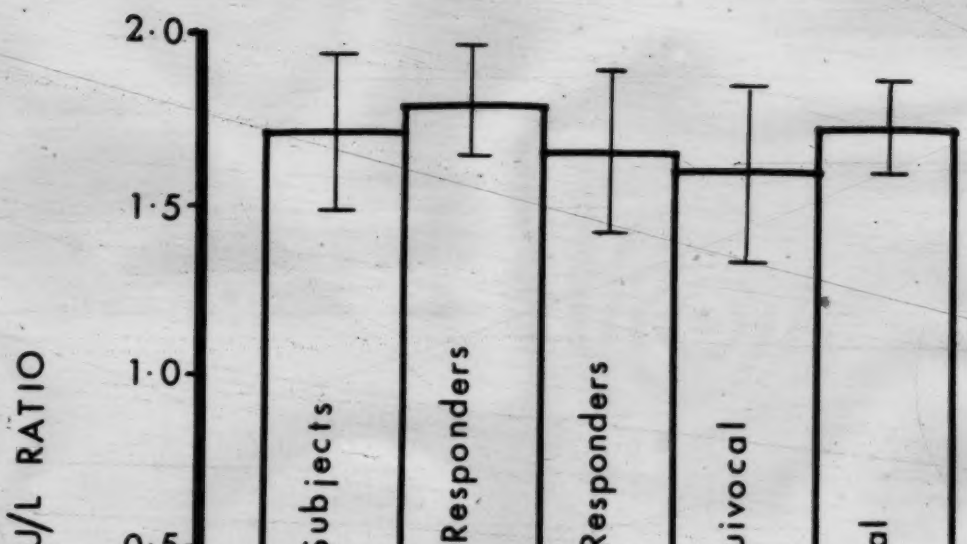


Fig. 31 - Initial U/L ratio for all subject groups (data from Table 8). Mean ± 1SEM.

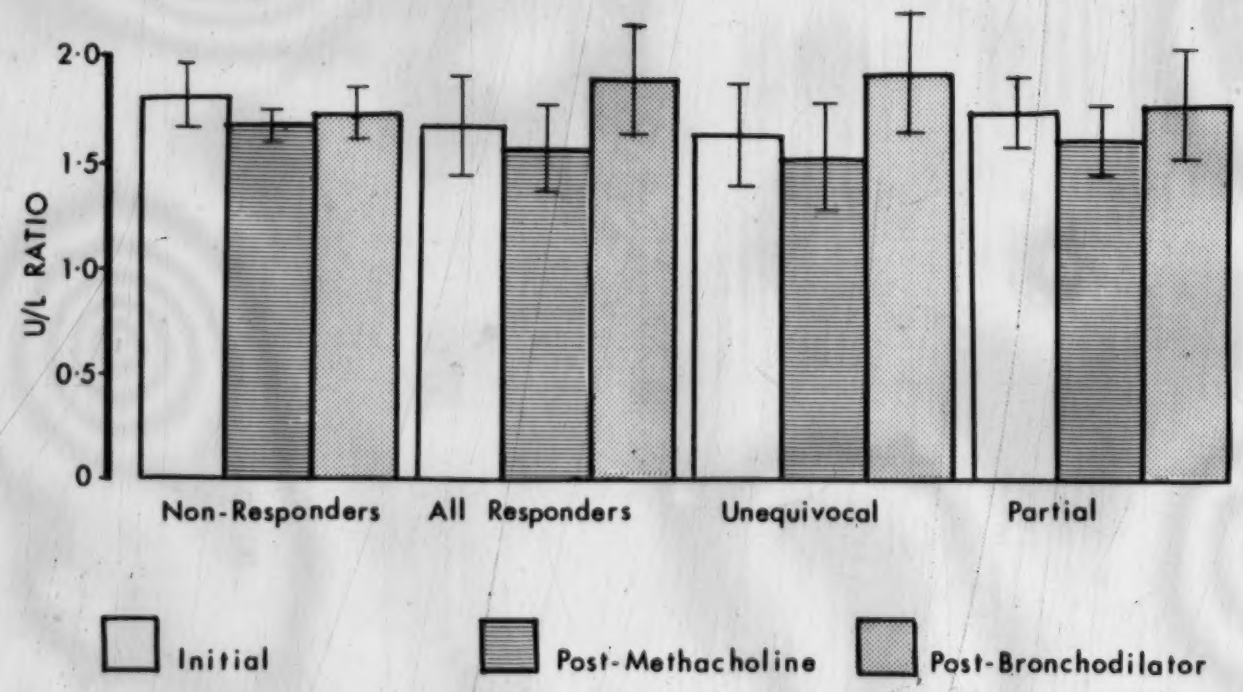


Fig. 32 - Change in U/L ratios following methacholine and bronchodilator inhalation (Data from Table 10). Mean ± 1SEM.

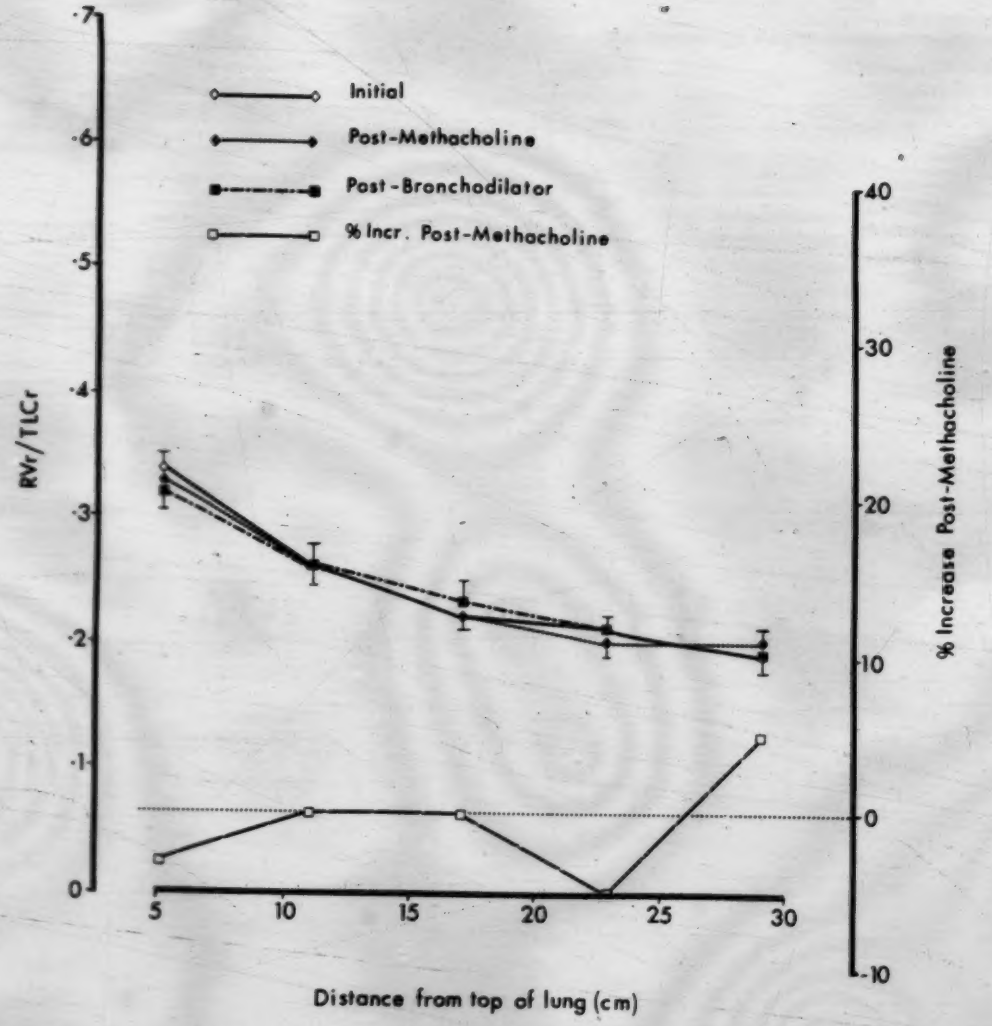


Fig. 33 - Change in RVr/TLCr values for all nonresponders following methacholine and bronchodilator inhalation (Mean ± 1SEM). Note the position of the origin of the right-hand Y axis differs from that in figures 34-36.

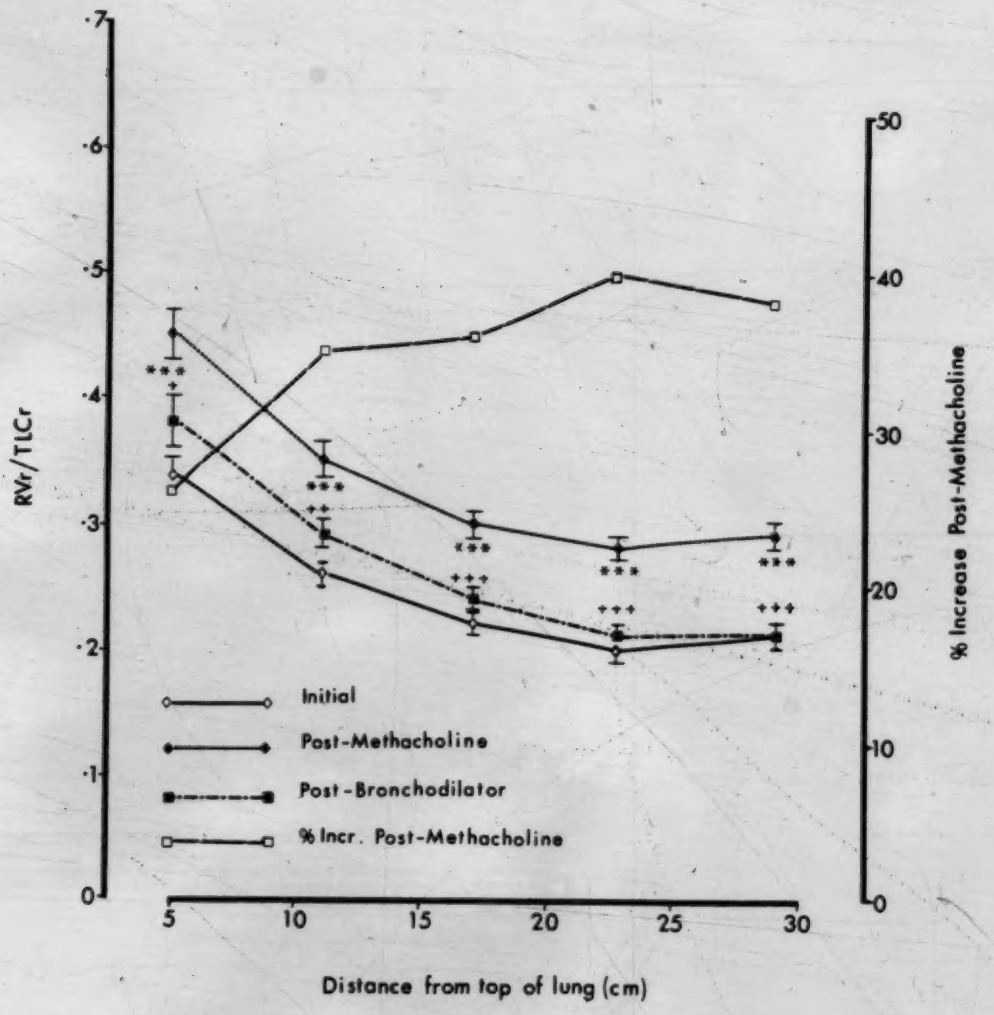


Fig. 34 - Change in RVr/TLCr values for all responders following methacholine and bronchodilator inhalation (Mean ± 1SEM).

***p<.001 for regions indicated comparing initial with post-methacholine results.

+p<.05; ++ p<.01; +++p<.001 for regions indicated comparing post-methacholine with post-bronchodilator.

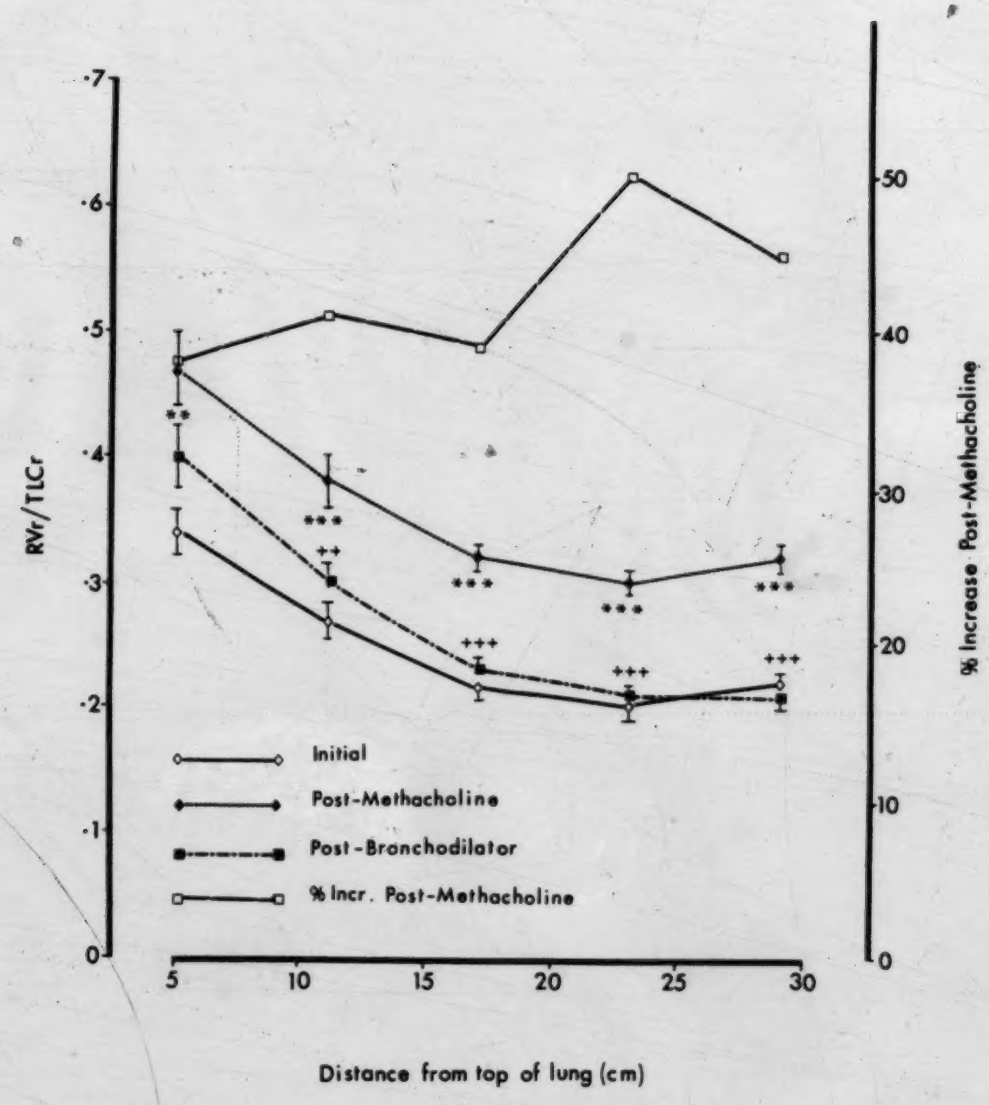


Fig. 35 - Change in RVr/TLCr values for "unequivocal responders" following methacholine and bronchodilator administration (Mean ± 1SEM).

***p<.001; **p<.01 for regions indicated comparing initial with post-methacholine results.

+++p<.001; ++p<.01 for regions indicated comparing post-methacholine with post-bronchodilator.

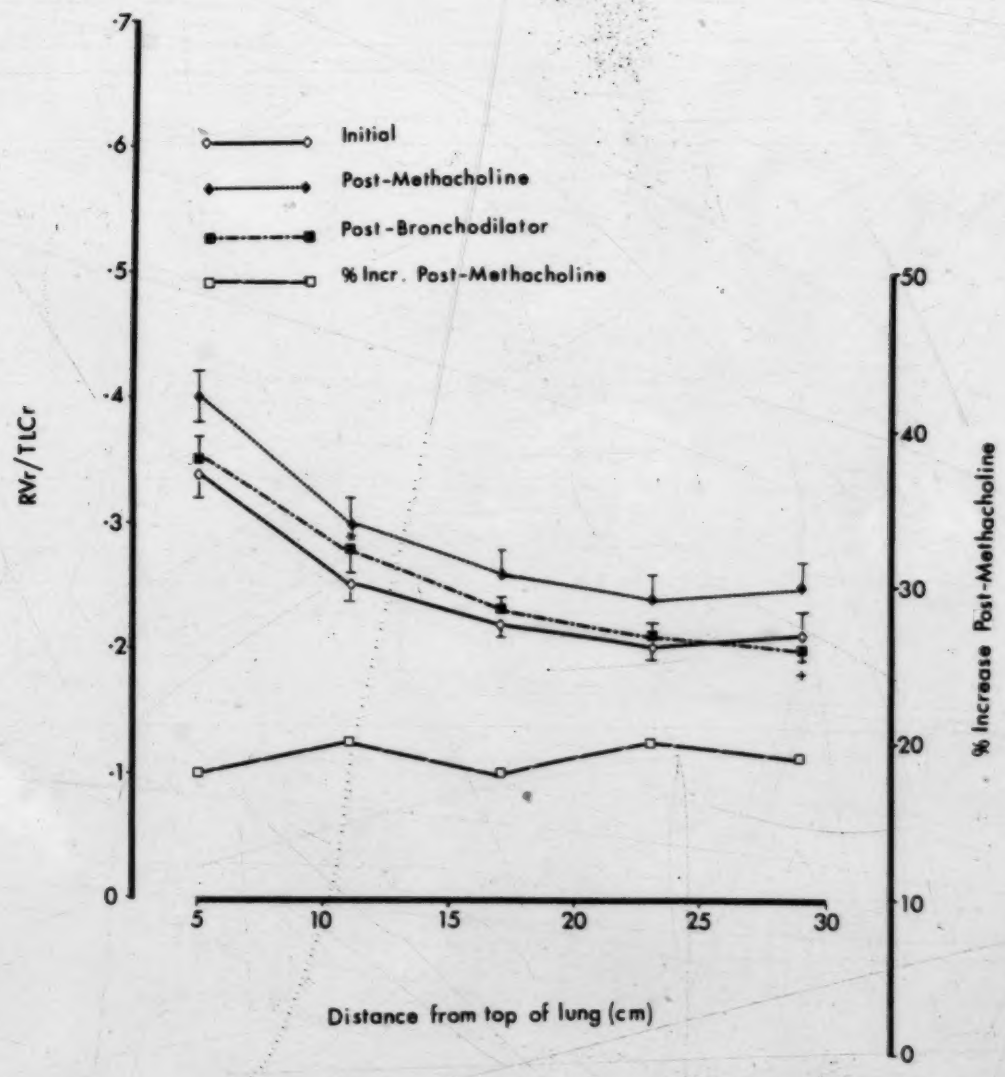


Fig. 36 - Change in RVr/TLCr values for "partial responders" following methacholine and bronchodilator administration (Mean ± 1SEM). Note position of origin of the right-hand Y axis differs from figures 33-35.

*P<0.05 for region indicated comparing initial with post-methacholine results.

+P<0.05 for regions indicated comparing post-methacholine with post-bronchodilator results.

Chapter V

DISCUSSION

Regional residual volume increased significantly in subjects who were responsive to bronchial provocation with methacholine but was not altered in nonresponsive individuals. The RVr/TLCr measurements provide sensitive indicators of mild degrees of methacholine sensitivity, being increased in subjects who showed only partial responsiveness with decreases in $\dot{V}_{50}$, $\dot{V}_{75}$ or isovolume flow rates without significant corresponding falls in FEV_1.

Pulmonary Function Data

Interpretation of normality of pulmonary function test results is subject to considerable variability between physicians. Cary et al (137) found agreement in only 58% of reports on the presence and degree of airways obstruction of 10 patients when their lung function data was reported independently by 26 pulmonary physicians. Variability of the patient's performance of pulmonary function testing is also well recognised, the time of day and the timing in relation to the last meal being important considerations. Measurements of RV, for example, may be expected to be higher and FRC lower shortly after a meal than later in the day due to the full stomach displacing the diaphragm. Patient fatigue is also another important consideration, as is their level of motivation and cooperation.

Use of the 95% confidence limits for the standard error of the estimate (1.645 X SEE) mean that only 5% of true normals will be classified wrongly. The confidence limits

are not parallel to the regression line used for prediction of normal values, however, thus still do not provide the perfect solution to the problem of identifying normality clearly and objectively (138).

All except subject 2 reported in this study were lifelong nonsmokers (subject 2 had stopped smoking 10 years previously) and most did not fall by more than 1.645 X SEE below their predicted values. Subject 2 had evidence of mild airflow obstruction, with FEV_1 67% of VC, impairment of end-expiratory flow rates (ET-FEV_1, $\dot{V}_{50}$, $\dot{V}_{75}$) and elevation of RV. The closing capacity measurement in subject 14 was elevated in association with decreased end-expiratory flow rates. Overall, however, there was no significant difference in the pulmonary function data of the responders and nonresponders when compared by unpaired Student's t-testing. The greater bronchodilator responsiveness of the responders is reflected in their larger standard deviation around the mean, subject 9 showing a particularly substantial increase in $\dot{V}_{50}$ and $\dot{V}_{75}$ after bronchodilator.

The nonresponders to methacholine could not be clearly differentiated from the responders by pulmonary function data alone. Their standard deviations around the means for most of the tests performed were slightly smaller than in the responder group, but all showed an increase in end-expiratory flow rates following bronchodilator. Thus bronchodilator responsiveness, as an indicator of increased bronchomotor tone, cannot separate out those subjects likely

to respond to nonspecific challenge testing from those who will not.

The body box determination of RV and FRC presents difficulty for some individuals, a consistent respiratory rhythm and effort being necessary to adequately determine the α angle (See Chapter III). Nine subjects had RV values in the 190-260% of predicted range. Three of these subjects were nonresponders (subjects 21,24,26) and one of the others (subject 12) found difficulty with the synchronisation required in the body plethysmograph determination of FRC.

The diffusion capacity is an extremely valuable test in the assessment of small airways function. Zamel recommends it as "probably the most indispensable simple test of pulmonary function and an extremely useful test of the real silent zone of the lungs" (139). Subject 2 again had the greatest degree of impairment of this measurement, although still achieving 73% of his predicted value. All other subjects were close to, or exceeded, their predicted values.

Skin testing is a convenient, objective way of assessing atopy. Eight of the 11 "unequivocal responders" tested showed positive results, 4 being strongly positive, and 2 of the "partial responders" showed mild to moderately positive tests. One of the nonresponders, however, had a moderately positive response despite no previous history to suggest atopy and another had a significant itchy reaction to 3 grass pollens tested and to mites although without weals developing. Thus, whilst the presence of positive

skin testing makes a subject far more likely to be a responder to inhaled methacholine than a nonresponder, even this does not provide an absolute method of discrimination prior to challenge testing, three "unequivocal responders" having negative skin tests.

Methacholine Challenge

Increased airways reactivity was confirmed in all except one (i.e. 14 out of 15) of the subjects with respiratory symptoms. The degree of sensitivity to methacholine varied quite considerably between patients, not clearly corresponding to the severity of the symptoms. Subjects 13,14 and 16, for example, were only classed as "partial responders" despite respiratory symptoms at the time of study. Four volunteers had evidence of increased nonspecific airways reactivity, although 2 were only mildly sensitive to methacholine ("partial responders" 15 and 17).

The isovolume flow rates provide the most sensitive index of methacholine sensitivity. Isovolume $\dot{V}_{75}$ was below 50% of control $\dot{V}_{75}$ in all "unequivocal responders", falling to 0% of control in 5 of these subjects. Interestingly, in 5 of the 8 nonresponders, the $\dot{V}_{75isovol}$ was reduced to 50-70% of control $\dot{V}_{75}$ without any corresponding change in regional lung function (see below). All the "partial responders" also showed a 25% or greater fall in $\dot{V}_{75isovol}$. Isovolume $\dot{V}_{50}$ was significantly reduced (>25%)

in all "unequivocal responders" but only 3 out of 6 "partial responders", as compared with 4 out of 8 nonresponders (although 2 of these were only just below 75% of control $\dot{V}_{50}$ measurements).

In terms of therapeutic implications it is most important to differentiate those with substantially increased airways reactivity, and thus likely to benefit most from bronchodilators, from those with normal or only slightly increased reactivity. The PC_{20FEV_1} appears to be the most useful indicator, only 2 "unequivocal responders" requiring the maximum concentration of 25 mg/ml methacholine to produce a 20% fall in FEV_1. By this criteria, only 2 healthy volunteers were demonstrated to be sensitive to methacholine, one with an atopic history (subject 7) and one nonatopic and entirely asymptomatic (subject 5).

The complete challenge test (without regional lung function studies) generally took 50-60 minutes to finish. This included the time necessary to instruct the patient on breathing pattern and 10-15 minutes delay after bronchodilator administration to ensure complete reversal of induced bronchoconstriction or symptoms. Even in the subjects experiencing the greatest degree of bronchospasm following methacholine inhalation (subjects 1 and 9) the effects were readily reversed by 2 puffs of Salbutamol from a standard pressurised cannister aerosol. It would therefore seem reasonable to propose an abbreviated challenge test, similar to that described by Chatham et al

(140), using only 5 mg/ml and 25 mg/ml methacholine solutions. In their recent paper they showed excellent correlation between the results of their short challenge test and conventional challenge testing, completing the short challenge in 6-12 minutes. A single deep breath of 5 mg/ml solution is inhaled followed, after spirometry, by 4 further breaths of this solution if no change occurs. If repeat spirometry then remains unchanged the same procedure is repeated with 1 and then 4 breaths of 25 mg/ml methacholine solution. Their choice of concentrations appears reasonable in the light of the data reported in this thesis, since 7 of the 12 "unequivocal responders" reacted to 5 mg/ml methacholine or less and the most sensitive subject (subject 1) would therefore be expected to react to a single breath of 5 mg/ml solution.

The distinction between airways sensitivity and reactivity has been mentioned in Chapter III (page 88). The two appear to assess different aspects of the airways response to nonspecific challenge, Orehek et al being unable to demonstrate a significant correlation between the two measurements (129). From the data reported here it is clear that those subjects who responded at the lower concentrations of methacholine had not only lower PC_{20FEV_1} values but also higher degrees of airways reactivity. There is therefore a division within the group of "unequivocal responders". Those requiring maximal doses of methacholine (25 mg/ml or 215 cumulative dose units) before a 20% change

in FEV_1 was observed (subjects 2 and 6) were obviously much less sensitive (i.e. had less reactive airways) to methacholine than those reacting at lower doses. The rate of decrease in FEV_1 with increasing methacholine doses (i.e. the slope of the dose-response curve, indicating reactivity) would therefore appear to be directly correlated with the PC_{20FEV_1} (or PD_{20FEV_1}). Interpretation of the slope, however, requires standardisation of the method of data plotting, as well as being dependent on the mode of aerosol delivery used. The simpler reporting of a PC_{20FEV_1} value makes this preferable at present, a graphic plot of the dose-response curve supplementing this information. If the area under the dose-response curve were to be calculated, as suggested by Townley at el (7) who used a parabolic curve-fitting analysis by the method of least squares, this would increase the complexity of the test without providing any extra useful information.

The Effect of Methacholine on RVr/TLCr

The subjects have been grouped according to both the effect of methacholine on spirometry and on RVr/TLCr. The maximum effect on RVr/TLCr was observed in the subjects with the greatest sensitivity to methacholine (the "unequivocal responders"). No effect at all was observed in the 8 nonresponders despite changes in $\dot{V}_{75isovol}$ in 5 of these subjects, as discussed above. A small increase in RVr/TLCr followed after methacholine inhalation in the "partial

responders" who had a 25% or greater decrease in $\dot{V}_{50}$, $\dot{V}_{75}$ or isovolume flow rates. This increase was only statistically significant at the 5% level for the lung zones 11 cm from the tops of the lungs, as shown in Fig.36.

The consistency of the RVr/TLCr measurements made on the 8 nonresponders indicates that it was possible to reposition subjects accurately for the three separate RVr/TLCr studies. Small changes in the depth of inspiration to TLC would therefore not have any major effect on RVr/TLCr measurements, the same zones being studied at TLC on each of the three occasions. Similarly reduction in FVC induced by methacholine inhalation in responders would not significantly affect TLCr, only RVr, and it is therefore reasonable to accept that the same lung zones were viewed by the scintillation detectors in the 3 studies. In some short subjects (subject 18, for example) the basal detectors were positioned too low and recorded higher RVr/TLCr measurements than in the zones immediately above them. This is one of the limitations of a fixed detector system, the centers of the lowest detectors always being 29 cm below the tops of the lungs.

A patchy change in RVr/TLCr, due to an increase in RVr, was demonstrated in the subjects responsive to methacholine. This was true for the "partial responders" as well as the "unequivocal responders", although this is not evident from the mean values (Tables 5 and 6) and the magnitude of changes for the "partial responders" were much

less. Thus, by taking the mean values alone, some information is lost about the pattern of induced bronchospasm.

The same method of aerosol delivery, with inspiratory capacity breaths from FRC, was used for all subjects, all tests being carried out by a single investigator. Other workers have shown that in this way a consistent level of dose delivered and distribution of inhaled aerosol may be achieved, both from breath to breath and between patients (34,35,39,45). If the aerosol is delivered too early in inspiration, when the inspiratory flow rates are high, the majority of the aerosol is deposited in the proximal, larger airways (39). This could therefore occur if the subject did not breathe in as soon as the indicator light appeared on the dosimeter. On average an adult can inhale comfortably from FRC to TLC in around 0.6 seconds (34), thus the 0.5 second delay after the indicator light appears prior to aerosol delivery makes allowance for a slight delay in the onset of inspiration. In fact, after preliminary instruction and with constant encouragement throughout the challenge testing (with particular emphasis on the correct timing of each phase in the respiratory cycle), no subject found difficulty in maintaining the correct mode of inhalation throughout the test.

A central pattern of deposition, resulting in airflow obstruction predominantly in the major airways, would affect airflow but not RVr/TLCr since this depends on airway

closure (i.e. small airways). Since the aerosol delivery technique favours peripheral deposition in the airways, test of small airways function would be expected to become abnormal before the FEV_1. This was observed in all the subjects studied, including some of the nonresponders who showed changes in the isovolume flow rates, $\dot{V}_{50}$ or $\dot{V}_{75}$ without any corresponding FEV_1 or RVr/TLCr changes. It is possible that, in the nonresponders who showed $\dot{V}_{75isovol}$ changes, the airways affected by the methacholine were not small enough to cause an increase in RVr/TLCr. A greater degree of change in end-expiratory flow rates was observed in the "partial responders" with RVr/TLCr changes than in those nonresponders who had changes in $\dot{V}_{75isovol}$. The greatest change in $\dot{V}_{50}$, $\dot{V}_{75}$ and isovolume flow rates occurring in the "unequivocal responders" with the highest degree of methacholine sensitivity.

Pulmonary ventilation is sometimes unevenly distributed in patients with spasmodic asthma, even during periods when they are symptom-free (1,108,109,141). The lung bases were involved most frequently and the middle zones least frequently in one study (11), whereas a previous study in this Department showed the mid-zones to be most severely affected (108). Methacholine causes bronchoconstriction through its action on bronchial smooth muscles, leading to increased airways resistance and consequent air trapping distal to the site of obstruction (Fig. 37). From the results obtained in this study it is seen, therefore, that

similar changes may be induced with methacholine as occur in "true" asthma.

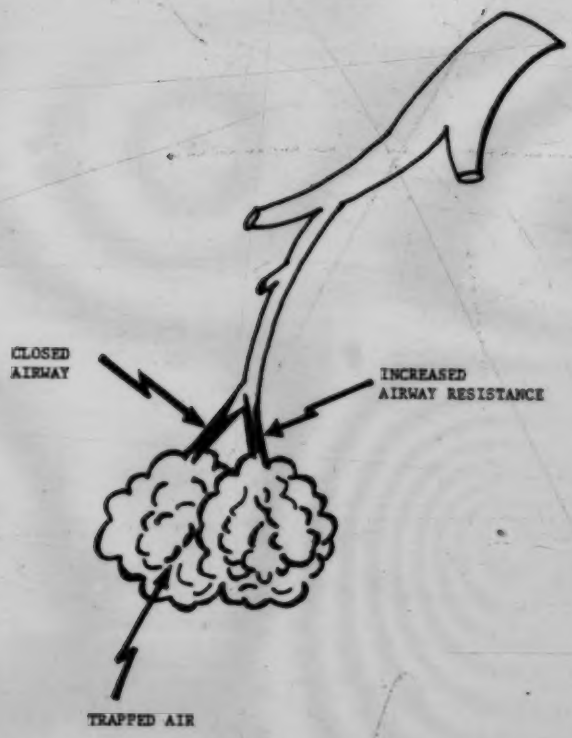


Fig. 37 - The effect of inhaled methacholine.

Overall the tendency was for a greater percentage increase in RVr/TLCr in the lower lung zones (Figs. 34 and 35). These mean values, however, reflect the average figures from some subjects with generalised increases, some with predominantly upper zone increase and others with mainly lower or mid-zone changes (Table 14, Appendix E). As demonstrated in Figs. 26-29 some lung zones may not show any

increase at all following methacholine inhalation despite adjacent zones being considerably affected. The severity of the changes also differed between the left and right lung of individual patients in all subjects studied.

The study by Engel et al (8) demonstrated a significant decrease in the U/L ratio from 3.21 ± 0.33 (mean ± 1SEM) to 1.27 ± 0.12 after methacholine, returning to 3.89 ± 0.85 after bronchodilator in seven seated normal subjects aged 24-36 years. Figure 32 shows, however, that in the 26 subjects reported in this thesis, the reduction in upper to lower zone ratios following methacholine was minimal and not statistically significant. The greatest change, in fact, occurred in the eight nonresponders, the mean U/L ratio changing from 1.79 to only 1.65 following methacholine.

The mean absolute difference in RVr/TLCr measurements recorded between the upper and lower zones studied decreased after methacholine in four out of five of Engel's subjects from 0.22 to 0.11 (8). The vertical gradient of RVr/TLCr measurements was therefore seen to be reduced following methacholine in their study. Study of the data in Tables 4 and 5 does not confirm these findings in the 26 subjects reported. The mean absolute differences between upper and lower zone RVr/TLCr ratios was initially 0.14 ± .05 (mean ± 1SD) in the nonresponders and 0.13 ± .04 following methacholine with no further change following bronchodilator. "Unequivocal responders" had an initial difference of 0.14 ± .05, 0.17 ± .14 after methacholine and

0.19 ± .11 following bronchodilator, not representing statistically significant changes. "Partial responders" had a difference of 0.16 ± .05 initially and 0.14 ± .06 following methacholine and .16 ± .07 bronchodilator. The responders as a whole (subjects 1-18) showed a slight increase in upper to lower zone difference from 0.15 ± .08 initially to 0.16 ± .12 following methacholine and 0.18 ± .10 after bronchodilator. Thus the overall tendency was for a slight increase in the difference between upper and lower zone RVr/TLCr values following methacholine in the "unequivocal responders", with a further slight increase after bronchodilator. The "partial responders" and nonresponders showed negligible changes in these values, no statistical significance being associated with the changes in any of the subject groupings.

There are three possible explanations for these differences from Engel's results. Firstly, 15 of the subjects reported in this thesis were referred because of respiratory symptoms and therefore may have behaved differently from "normals" in response to methacholine. Eleven subjects, however, had no respiratory symptoms, 7 of these being methacholine nonresponders, 2 "unequivocal responders" and 2 "partial responders". No significant difference existed between initial lung function or RVr/TLCr data between the different subgroups of subjects. The pulmonary function data used to confirm "normality" is not reported by Engel et al (8) but all of their seven "normal"

subjects were methacholine responsive. It is therefore difficult to argue that their subjects were any different from the larger series reported here. The fact that all seven of Engel's subjects responded to methacholine suggests that their sample was not representative of the normal population and that all seven had increased bronchomotor tone (as also suggested in their paper by the effect of Isoprenaline inhalation) and thus were not truly "normal".

The second, probably most important, explanation is that the inhalational techniques were different. In this study a method of intermittent aerosol generation has been used with controlled inspiratory capacity breaths, favouring peripheral aerosol deposition throughout the lungs. Engel et al administered larger doses of methacholine (100 mg/ml) by a continuous aerosol generation technique (Hudson nebuliser at 5 ℓ/min flow) for 30 seconds. If the bronchoconstriction induced (as assessed by FEV_1) wore off before repeating the ^{133}Xe study, a further dose of methacholine was administered. Aerosol is generated throughout inspiration and, during tidal breathing, Potchen and Evens (142) have demonstrated that this leads to greatest ventilation (and hence presumably aerosol deposition) in the lower lung zones. This is probably due to the greater distensibility of alveoli in these regions during tidal breathings (79,101,102) as outlined when considering the determinants of regional lung volume (p.34). The exact pattern of deposition produced by

intermittent aerosol generation techniques has not been documented in the literature. It seems likely, however, that the U/L ratio differences between this study and that of Engel et al (8) can be explained by different sites of aerosol deposition for the different techniques.

A third factor to consider is the difference in ^{133}Xe techniques employed. A bolus of ^{133}Xe is preferentially distributed to the lung apices when inhaled from RV up to TLC (101). This method therefore employs the same principles as the single breath N_2 washout study introduced by Fowler (77). A slow exhalation back to RV permits measurement of ^{133}Xe concentration in exhaled air. Engel et al combined this technique with a ^{133}Xe equilibration procedure (to give TLCr) to calculate RVr/TLCr. The RVr measured was then corrected for the different chest geometry viewed by a set of 5 paired posterior detectors following exhalation to RV from TLC. The necessity to adjust observed results in this way may introduce a potential source of error. This is avoided by the technique employed in the study reported in this thesis, using paired colinear anterior and posterior detectors with measurements made at a standardized lung volume (i.e. TLC).

Whilst the results of this study differ from those of Engel et al (8), the conclusions reached are in agreement. The methacholine responsive individuals showed a patchy change in RVr/TLCr indicating that there is not a single critical closing pressure (i.e. point at which the closing

forces of bronchoconstriction exceed the airways opening pressure) for all airways. In some individuals there appears to be an increased upper zone predisposition to airways closure following methacholine inhalation, resulting in a greater percentage change in the upper than the lower lung regions and therefore an increased U/L ratio in these subjects. Similarly, other subjects were seen to have greater lower and mid-zone changes, as observed by Engel et al (8), and in those individuals there was a reduction in the U/L ratio. Others showed a generalised increase in RVr/TLCr which did not alter the U/L ratio.

Since some subjects gave a history to suggest episodic bronchospasm it is conceivable that there may have been impaired lung function prior to challenge testing. Pre-existant airways obstruction is well recognised as causing regional ventilatory impairment (108) thus this was an important exclusion in these subjects. All initial RVr/TLCr ratios were within the normal ranges (104). This therefore does not appear to have been a significant factor in explaining why there were some subjects who had no change in RVr/TLCr in some lung zones despite large changes in adjacent lung zones. This observation does, however, add further support to the concept of different critical opening and closing pressures of alveoli in different lung regions.

Changes in RVr/TLCr were observed in all lung zones in some subjects, thus it may be concluded that the receptors involved in bronchospasm are distributed diffusely

throughout the airways as in the cat (39,73). It is also apparent that Moll's concept of the pathogenesis of airways sensitivity to methacholine was incorrect (20) and that, based on currently available spirometric and isotope techniques to assess lung function, pre-existent lung "damage" is not necessary for methacholine sensitivity to occur.

The Effect of Inhaled Bronchodilator Following Methacholine Inhalational Challenge

As mentioned in the preceding section, Engel et al (8) observed that Isoprenaline administered following methacholine-induced bronchoconstriction restored the upper to lower zone RVr/TLCr ratio to normal (3.21 ± 0.33 [SEM] initially, 1.27 ± 0.12 post-methacholine and 3.89 ± 0.85 after Isoprenaline). Isoprenaline administered without prior methacholine inhalation led to a significant increase in U/L ratio from 3.23 ± .47 to 5.49 ± .85. Isoprenaline alone did not change the absolute difference between upper and lower zone RVr/TLCr measurements. Jones et al (108) showed that inhaled bronchodilator could significantly improve regional ventilation, as assessed by regional ventilation at increased respiratory frequencies.

In the study reported here the mean RVr/TLCr ratios post-bronchodilator were not significantly different from the initial measurements. In several subjects, however, the reversal of induced RVr/TLCr changes were incomplete

following bronchodilator despite having waited until spirometry (including $\dot{V}_{50}$, $\dot{V}_{75}$ and isovolume flow rates) had returned to, or exceeded, control values. This indicates that tests of regional lung function may, in some circumstances, be more sensitive than spirometric tests of the small airways. Another explanation for this observation is that the two tests are in fact measuring two different things - flow rate by spirometry and the effect of small airways closure by RVr/TLCr. The results also demonstrate that regional ventilatory impairment may exist without spirometric evidence of bronchospasm, as described in other studies (11,12,108,141). The U/L ratio increased slightly in all subjects following Salbutamol, exceeding initial measurements in all responders but not nonresponders (Fig.32).

The increased U/L ratio post-bronchodilator in the "unequivocal responders" was significant at $p<.001$ when compared to the post-methacholine values and $p<.05$ compared to the initial measurements. For responders as a whole (subjects 1-18) the increase in U/L ratio post-bronchodilator compared to the post-methacholine value was significant at $p<.05$. The changes post-bronchodilator in the "partial responders" and nonresponders did not differ significantly from initial or postmethacholine values.

The absolute difference between mean upper and lower zone RVr/TLCr measurements increased slightly in the "unequivocal responders" group (0.14 ± .05 initially,

0.17 ± .14 post-methacholine and 0.19 ± .11 post-bronchodilator). There was no change in this numerical difference post-bronchodilator compared to post-methacholine in the "partial responder" or nonresponder groups. This observation differs from that of Engel et al (8), who showed a significant increase in both the U/L ratio and absolute different between upper and lower RVr/TLCr post-bronchodilator in their subjects. Those subjects with the greatest level of airways reactivity, the "unequivocal responders", had the greatest increase in the difference between upper and lower RVr/TLCr, suggesting the most labile bronchomotor tone. They would therefore react most ready to airways irritants (iatrogenic or environmental) and also be expected to benefit most from an inhaled bronchodilator.

There was a tendency for the reversal of RVr/TLCr changes after bronchodilator to be least complete in the upper lung zones than at the lung bases (Figs. 34-36). Although this apical difference was not significantly different from the initial RVr/TLCr values, the observation was a little surprising since, when inhaled from RV, an aerosol bolus will first be preferentially distributed to the lung apices (110). The explanation for this apparent discrepancy may simply be that pressurised cannister aerosols are inefficient (45), only 3% of the dose reaching the alveoli. A better explanation is that, if the aerosol cannister is activated relatively late in inspiration, less aerosol will go to the apices and more to the lower lung regions.

Chapter VI

CONCLUSIONS

When a patient presents with a history of episodic respiratory symptoms (cough, wheeze, dyspnea, chest tightness, etc.) it may be difficult to clearly document the etiology if physical examination and routine tests are normal. This study has clearly demonstrated the value of methacholine challenge testing in determining the degree of nonspecific airways reactivity, separating out subjects with an asthmatic level of reactivity from those with a lesser degree of sensitivity to methacholine. A significantly increased level of airways reactivity was demonstrated in 10 of the 15 patients referred for challenge testing, 4 of the remaining 5 having a lesser degree of sensitivity to methacholine.

Despite normal basline data (spirometric and ^{133}Xe measurements of RVr/TLCr) for all subjects, the effect of inhaled methacholine on RVr/TLCr differed considerably between subjects. Those subjects with no spirometric evidence of methacholine response showed no change in RVr/TLCr following methacholine and those with only "partial" responsiveness ($\dot{V}_{50}$, $\dot{V}_{75}$, isovolume flow rates) showed only small increases in RVr/TLCr. The most striking change in RVr/TLCr measurements were observed in the "unequivocal responders" (PC_{20FEV_1} achieved), who exhibited patchy changes in regional lung function.

Since initial RVr/TLCr measurements were normal it seems unlikely that significant airways obstruction (and hence impaired regional ventilation) existed in those

regions which were unaffected by inhaled methacholine. Some subjects showed maximum increases in RVr/TLCr at the lung bases, whilst others had greater changes in the upper or mid lung zones. The degree of increase in RVr/TLCr was not the same in corresponding lung zones of the left and right lungs for individual patients. These findings support the concept of independent critical opening and closing pressures for airways in different regions of the lungs (8,110). They also demonstrate that methacholine induced bronchospasm simulates true asthmatic bronchospasm, both in induced regional ventilatory impairment as well as in spirometric abnormalities. The changes may be reversed readily with inhaled bronchodilator, although reversal may be incomplete when assessed with ^{133}Xe RVr/TLCr measurements despite return to baseline (or better) of spirometric results.

The airways receptors responsible for causing bronchoconstriction appear to be distributed diffusely through the lungs, since either specific or all of the 10 lung regions assessed in this study may be affected by inhaled methacholine. Pre-existent lung damage, as suggested by Moll (20), clearly need not necessarily be present for a bronchoconstrictor response to methacholine to occur.

Most of the previous studies of aerosol deposition have been performed using continuous aerosol generation techniques. It would therefore be of interest to further evaluate the deposition pattern with the intermittent

aerosol generation technique used in this study, using methods similar to those of Newhouse and Ruffin (39). This may show why such a patchy alteration in RVr/TLCr values was observed and also help explain the differences between the post-methacholine U/L ratios found in this study and those reported by Engel et al (8). Another benefit from further isotope deposition studies would be in determining the most efficient method for aerosol delivery to obtain optimal peripheral airways penetration. Techniques of aerosol delivery for challenge testing must necessarily be quick and simple if they are to be of value as routine clinical tests, intermittent aerosol generation methods probably being the best in this regard (35).

A major problem in the assessment of airways reactivity is the lack of uniformity in techniques currently in use. Hopefully the ATS guidelines (35) will help achieve some standardisations but, whilst several methods of aerosol delivery are in use, it is difficult to relate results obtained in one center to those from another. This is particularly true in the determination of airways reactivity from the slope of the dose-response curve, there being no uniformity regarding the exact method of plotting the data points or of measuring the slope. Airways sensitivity to methacholine is easier to compare in the literature, but clearly much valuable information about the pattern of bronchial responsiveness to inhaled irritants is being lost through this inconsistency of technique.

If we are really to learn about the pathogenesis of airways reactivity, perhaps Professor Woolcock is right in that measurement of nonspecific airways reactivity should become

> "as essential to the diagnosis and management of asthma as the glucose tolerance test is to diabetes" (31).

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APPENDICES

APPENDIX A

METHACHOLINE CHALLENGE AND REGIONAL RV/TLC RATIOS

CONSENT FORM

Methacholine challenge testing is a method of assessing how sensitive your airways are by asking you to inhale serial concentrations of methacholine solutions. The test ends when a 20% fall in your initial breathing test result (FEV_1) is observed. Mild breathlessness may be produced, but this is readily corrected with Ventolin inhalation.

So that we may get further information about how your lungs react during the test, three studies of your regional lung function will also be made using inhaled 133-Xenon gas. No injections are required. The changes in lung function are detected by having you seated between special detectors which give a plot of your lung volumes (which are proportional to the amount of Xenon in your lungs at a given time). The overall amount of radiation exposure is comparable to a set of routine chest x-rays.

Any female subject who is pregnant should inform the supervising physician prior to starting the test, as Xenon scans will not be used in this case.

All test results are confidential and your anonymity will be preserved in publication of the results of this study.

I, _____, have read the above information and have had the study explained to me by Dr. Horsley. I consent to participation in this study and understand that I may withdraw from the study at any stage during the testing.

Signed
Date _____

Witness _____

Dr. J.R. Horsley, MB, ChB. _____

APPENDIX B

TABLE 9

DISTRIBUTION OF SUBJECTS BY AGE, SEX, PHYSICAL CHARACTERISTICS AND SYMPTOMATOLOGY

ID#	Sex	Atopy	Age (yrs)	Height (cm)	Weight (kg)	Symptom
a) Responders						
1.	F	+++	20	173	84.5	a,b
2.	M		50	169	64.5	a,c
3.	M	++	30	176	75.0	a,b,c
4.	F	+++	33	161	63.5	a,b,d
5.	F	-	26	157	54.0	e
6.	M	+	21	170	71.0	a
7.	F	+++	25	176	62.5	e
8.	M	-	63	166	79.0	c
9.	F	+++	27	164	64.0	a,b
10.	F	+	58	173	84.0	b,d
11.	F	-	40	160	64.0	a,d
12.	F	+	20	152	49.5	a,b
Mean 1-12			34.4	166	68.0	
SD 1-12			15.0	8	11.0	
13.	M	+	39	182	77.8	c,d
14.	M	++	37	175	74.0	b,c
15.	M	-	37	181	80.0	e
16.	F		32	164	72.0	a
17.	F	-	29	168	61.0	e
18.	F	-	44	152	56.0	c
Mean 13-18			36.3	170	70.1	
SD 13-18			5.3	11	9.6	

TABLE 9 cont'd

DISTRIBUTION OF SUBJECTS BY AGE, SEX, PHYSICAL CHARACTERISTICS AND SYMPTOMATOLOGY

ID#	Sex	Atopy	Age (yrs)	Height (cm)	Weight (kg)	Symptom
Mean 1-18			35.0	168	68.7	
SD 1-18			12.4	9	10.3	
b) Non-responders						
19.	F		29	172	53.5	e
20.	M	-	24	182	76.0	e
21.	F	-	31	166	63.0	e
22.	M	(+)	31	179	70.0	e
23.	M	-	29	184	67.0	e
24.	F	-	43	173	67.0	a
25.	M	++	25	182	82.0	e
26.	F	-	28	171	67.0	e
Mean 19-26			30.0	176	68.2	
SD 19-26			5.8	6	8.5	
Mean 1-26			33.5	170	68.5	
SD 1-26			11.0	9	9.6	

Sympton

a Dyspnea
b Episodic wheeze
c Cough
d Chest tightness
e Healthy Volunteer

Atopy (Skin tests)

- Negative
(+) Itchy
+ Induration 1-3 allergens
++ Moderate induration 3 - 6 allergens
+++ Strongly positive, 6 or more allergens

APPENDIX C

TABLE 10

PULMONARY FUNCTION DATA

		Subject			
		1	2	3	4
TLC (Box)	ℓ,% Pred.	6.28,119	7.81,133	7.15,111	5.32,119
TLC (He)	ℓ,% Pred.	5.42,103	7.05,120	6.51,101	4.27, 95
VC	ℓ,% Pred.	4.67,111	4.41,108	5.25,107	3.10, 90
FRC (Box)	ℓ,% Pred.	2.97,135	4.83,196	3.57,133	3.42,182
RV (Box)	ℓ,% Pred.	1.61,148	3.40,220	1.70,117	2.22,218
RV/TLC	% TLC	26	44	24	42
Trapped Air	ℓ	0.86	0.76	0.64	1.05
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	0.63,0.54	0.45,0.46	1.80,0.16	0.73,0.40
FIF	ℓ/S,% Pred.	5.67, 93	6.61,115	5.99, 88	5.02,125
FEV_1	ℓ,% VC	3.96, 85	2.94, 67	3.56, 68	2.55, 82
$ET\text{-}FEV_1$	ℓ,% ERV	1.06, 78	0.48, 34	0.70, 37	0.84, 70
FEF(25-75)	ℓ/S,% Pred.	4.15, 95	1.50, 37	2.63, 54	3.12,108
$\dot{V}_{50}$	ℓ/S,% Pred.	4.37, 98	1.63, 41	2.57, 54	3.25,107
$\dot{V}_{75}$	ℓ/S,% Pred.	2.52,113	0.43, 22	1.28, 53	1.48, 97
DLCO	ml/min/mmHg,% Pred.	32.1,116	16.8, 73	28.6, 95	25.9,118
CV	% VC	5.78			7.27
CC	% TLC	20.5			34.4
Slope N_2 washout	$\%N_2/\ell$	0.38			0.53
<u>After Bronchodilator</u> (Ventolin)					
VC	ℓ,% Pred.	4.70,111	4.58,110	5.14,105	2.99, 88
FEV_1	ℓ,% VC	4.22, 90	2.86, 63	4.11, 80	2.79, 94
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	0.52,0.73	0.85,0.26	0.67,0.42	0.54,0.66
$\dot{V}_{50}$	ℓ/S,% Pred.	4.32, 97	1.73, 43	3.75, 76	4.20,146
$\dot{V}_{75}$	ℓ/S,% Pred.	2.80,126	0.39, 19	1.45, 59	1.90,132

TABLE 10 cont'd

PULMONARY FUNCTION DATA

		Subject			
		5	6	7	8
TLC (Box)	ℓ,% Pred.	5.35,127		6.06,111	6.09,108
TLC (He)	ℓ,% Pred.	4.83,115	6.09, 99	5.44, 99	5.13, 91
VC	ℓ,% Pred.	3.62,107	5.37,111	4.45,105	3.65,100
FRC (Box)	ℓ,% Pred.	2.61,147	2.37, 91	3.17,138	3.47,146
RV (Box)	ℓ,% Pred.	1.73,195	0.97, 75	1.61,136	2.44,149
RV/TLC	% TLC	32	16	27	40
Trapped Air	ℓ	0.52		0.62	0.96
Raw $cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	1.64,0.23		1.39,0.23	0.61,0.48
FIF	ℓ/S,% Pred.	4.57, 97	5.88,102	5.60, 97	4.82,102
FEV_1	ℓ,% VC	2.63, 73	4.41, 82	3.23, 73	2.97, 81
ET-FEV_1	ℓ,% ERV	0.59, 67		0.77, 50	0.92, 89
FEF(25-75)	ℓ/S,% Pred.	2.49, 74	4.63, 79	3.07, 74	3.77,111
$\dot{V}_{50}$	ℓ/S,% Pred.	2.40, 69	5.60,116	2.97, 75	3.40, 99
$\dot{V}_{75}$	ℓ/S,% Pred.	1.00, 57	1.95, 76	1.18, 60	2.80,162
DLCO	ml/min/mmHg,% Pred.	22.0, 94	30.2, 95	27.9,103	26.4,117
CV	% VC	6.56	14.7		15.5
CC	% TLC	29.8	28.2		34.9
Slope N_2 washout,	$\%N_2/\ell$	1.15	1.60		1.85
<u>After Bronchodilator</u> (Ventolin)					
VC	ℓ,% Pred.	3.54,104	5.41,112	4.22, 99	3.68,101
FEV_1	ℓ,% VC	2.94, 83	4.67, 86	3.46, 82	3.34, 91
Raw $cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$			0.48,0.68	
$\dot{V}_{50}$	ℓ/S,% Pred.	3.00, 91	6.22,129	3.50, 89	5.90,120
$\dot{V}_{75}$	ℓ/S,% Pred.	1.40, 85	2.80,109	1.31, 66	2.80,107

TABLE 10 cont'd

PULMONARY FUNCTION DATA

		Subject			
		9	10	11	12
TLC (Box)	ℓ,% Pred.	5.24,112	6.17	6.07,138	4.83,125
TLC (He)	ℓ,% Pred.	5.01,107	5.19, 98	5.35,121	4.03,104
VC	ℓ,% Pred.	3.60, 98	3.29,105	4.02,123	2.92, 99
FRC (Box)	ℓ,% Pred.	2.78,142	3.15	3.05,164	3.02,185
RV (Box)	ℓ,% Pred.	1.64,162	1.50, 70	2.04,191	1.91,256
RV/TLC	% TLC	31	29	34	40
Trapped Air	ℓ	0.22	1.00	0.72	0.80
Raw $cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	0.95,0.38	0.68,1.13	0.61,0.53	2.03,0.16
FIF	ℓ/S,% Pred.	7.28,156	5.33,108	5.36,102	2.84, 75
FEV_1	ℓ,% VC	3.16, 88	2.57, 78	3.34, 83	2.14, 73
ET-FEV_1	ℓ,% ERV	0.77, 67		0.52, 52	0.79, 71
FEF(25-75)	ℓ/S,% Pred.	5.28,158	2.56, 67	4.35,116	1.69, 62
$\dot{V}_{50}$	ℓ/S,% Pred.	5.54,154	3.05, 70	4.90,131	1.82, 64
$\dot{V}_{75}$	ℓ/S,% Pred.	2.50,139	0.69, 28	1.73, 93	0.85, 60
DLCO	ml/min/mmHg,% Pred.	27.7,117	21.8, 96	30.4,131	28.3,121
CV	% VC	7.35	20.2	17.70	8.93
CC	% TLC	33.1	37.6	37.6	32.1
Slope N_2 washout,	% N_2/ℓ	2.39	8.14	1.96	2.87
After Bronchodilator (Ventolin)					
VC	ℓ,% Pred.	3.40, 92	3.39,108	3.55,109	2.95,102
FEV_1	ℓ,% VC	3.37 99	2.69, 97	3.14, 88	2.26, 77
Raw $cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	0.49,0.70		0.17,1.97	
$\dot{V}_{50}$	ℓ/S,% Pred.	6.92,201	3.42, 78	4.40,122	1.89, 67
$\dot{V}_{75}$	ℓ/S,% Pred.	3.30,191	2.56, 67	1.73, 96	1.05, 74

TABLE 10 cont'd

PULMONARY FUNCTION DATA

		Subject			
		13	14	15	16
TLC (Box)	ℓ,% Pred.	7.73,112	8.81,139	7.41,109	4.98,107
TLC (He)	ℓ,% Pred.	6.37, 92	8.60,135	8.02,118	4.29, 92
VC	ℓ,% Pred.	4.88, 97	5.88,125	5.52,110	3.35, 93
FRC (Box)	ℓ,% Pred.	4.56,158	5.30,199	4.31,151	2.57,131
RV (Box)	ℓ,% Pred.	2.85,171	2.93,194	1.90,117	1.64,155
RV/TLC	% TLC	37	33	26	33
Trapped Air	ℓ	1.36	0.21		0.69
Raw $cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	0.71,0.31	0.73,0.26	0.69,0.34	0.96,0.41
FIF	ℓ/S,% Pred.	5.65, 89	6.27, 82	4.46, 62	4.81,111
FEV_1	ℓ,% VC	3.97, 81	3.85, 66	3.88, 70	2.73, 81
ET-FEV_1	ℓ,% ERV	0.84, 49	1.10, 47	1.42. 59	0.60. 65
FEF(25-75)	ℓ/S,% Pred.	4.31, 95	2.46, 45	3.00, 58	3.15,101
$\dot{V}_{50}$	ℓ/S,% Pred.	3.38, 76	2.90, 54	2.30, 43	3.65,112
$\dot{V}_{75}$	ℓ/S,% Pred.	1.35, 61	1.03, 38	1.00, 37	1.55, 95
DLCO	ml/min/mmHg,% Pred.	30.5, 98	33.5, 89	39.7,111	23.4,102
CV	% VC		22.1	4.2	4.0
CC	% TLC		43.2	35.3	24.8
Slope N_2 washout,	$\%N_2/\ell$		2.18	0.64	1.90
After Bronchodilator (Ventolin)					
VC	ℓ,% Pred.	4.85, 97	5.80,124	5.55,111	3.50, 98
FEV_1	ℓ,% VC	3.97, 82	4.17, 72	4.11, 74	2.93, 87
Raw $cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	0.26,0.87	0.31,0.69		0.52,0.68
$\dot{V}_{50}$	ℓ/S,% Pred.	4.55,103	3.07, 57	2.90, 55	3.80,115
$\dot{V}_{75}$	ℓ/S,% Pred.	1.32, 60	1.09, 41	1.00, 38	1.65,100

TABLE 10 cont'd

PULMONARY FUNCTION DATA

		Subject			
		17	18	19	20
TLC (Box)	ℓ,% Pred.	5.13,105	4.70,124	5.93,114	7.34,106
TLC (He)	ℓ,% Pred.	4.83, 98	4.01,105	4.98, 96	6.21, 90
VC	ℓ,% Pred.	3.80,100	2.99,107	4.05,101	5.36,100
FRC (Box)	ℓ,% Pred.	2.76,134	2.12,132	3.73,171	3.73,129
RV (Box)	ℓ,% Pred.	1.33,123	1.72,178	1.89,163	1.97,132
RV/TLC	% TLC	26	36	32	27
Trapped Air	ℓ	0.30	0.70	0.96	1.13
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	0.75,0.48	1.31,0.36	1.04,0.26	0.29,0.94
FIF	ℓ/S,% Pred.	5.19,105	4.47,115	3.81, 72	8.39,120
FEV_1	ℓ,% VC	3.15, 83	2.34, 78	2.74, 68	4.03, 75
$ET\text{-}FEV_1$	ℓ,% ERV	0.99, 69	0.30, 55	1.24, 67	1.30, 74
FEF(25-75)	ℓ/S,% Pred.	3.95,112	2.87,103	2.61, 69	4.04, 81
$\dot{V}_{50}$	ℓ/S,% Pred.	4.30,121	2.90,105	3.00, 74	3.97, 80
$\dot{V}_{75}$	ℓ/S,% Pred.	1.40, 79	1.15, 79	1.20, 59	1.90, 77
DLCO	ml/min/mmHg,% Pred.	31.2,123	20.2,107	21.3, 88	43.2,131
CV	% VC		16.1	10.3	
CC	% TLC		36.7	26.7	
Slope N_2 washout,	$\%N_2/\ell$		1.54	3.54	
After Bronchodilator (Ventolin)					
VC	ℓ,% Pred.	3.61, 95	2.97,106	4.08,102	5.11, 96
FEV_1	ℓ,% VC	3.15, 87	2.55, 86	3.26, 80	4.32, 85
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	0.80,0.39	0.90,0.53		0.21,1.22
$\dot{V}_{50}$	ℓ/S,% Pred.	4.50,126	3.40,128	3.32, 84	5.30,110
$\dot{V}_{75}$	ℓ/S,% Pred.	1.75, 98	1.65,125	1.40,71	2.05, 85

TABLE 10 cont'd

PULMONARY FUNCTION DATA

		Subject			
		21	22	23	24
TLC (Box)	ℓ,% Pred.	6.71,140	7.88,118	7.67,109	6.43,122
TLC (He)	ℓ,% Pred.	5.54,115	6.42, 96	7.46,106	5.55,105
VC	ℓ,% Pred.	4.54,123	5.36,107	5.17, 97	3.93,104
FRC (Box)	ℓ,% Pred.	3.63,180	4.46,160	4.83,164	3.64,165
RV (Box)	ℓ,% Pred.	2.17,201	2.51,166	2.50,157	2.50,191
RV/TLC	% TLC	32	32	33	39
Trapped Air	ℓ	1.18	1.46	0.20	0.88
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	0.30,0.91	0.33,0.69	1.33,0.14	0.21,1.32
FIF	ℓ/S,% Pred.	5.40, 91	6.69, 96	7.87,117	4.72, 92
FEV_1	ℓ,% VC	3.61, 79	4.01, 75	4.11, 79	3.21, 82
$ET\text{-}FEV_1$	ℓ,% ERV	1.02, 70	1.37, 70	1.83, 78	0.81, 71
FEF(25-75)	ℓ/S,% Pred.	3.67, 87	4.42, 89	3.77, 78	4.54,124
$\dot{V}_{50}$	ℓ/S,% Pred.	3.85, 94	4.85, 93	3.30, 67	4.70,129
$\dot{V}_{75}$	ℓ/S,% Pred.	1.50, 73	1.57, 61	1.50, 61	1.60, 88
DLCO	ml/min/mmHg,% Pred.	26.0, 99	34.0,105	33.7, 94	26.8,102
CV	% VC	10.6	5.2	9.5	9.8
CC	% TLC	26.2	20.4	36.1	35.9
Slope N_2 washout,	$\%N_2/\ell$	2.87	2.49	1.08	1.08
<u>After Bronchodilator</u> (Ventolin)					
VC	ℓ,% Pred.	4.54,123	5.43,108	5.30, 99	3.79,100
FEV_1	ℓ,% VC	3.71, 82	4.39, 81	4.27, 81	3.26, 86
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$				
$\dot{V}_{50}$	ℓ/S,% Pred.	3.60, 87	6.10,120	3.80, 77	4.55,126
$\dot{V}_{75}$	ℓ/S,% Pred.	1.55, 75	1.50, 59	1.90, 77	2.30,128

TABLE 10 cont'd

PULMONARY FUNCTION DATA

		Subject	
		25	26
TLC (Box)	ℓ,% Pred.	8.71,126	6.85,134
TLC (He)	ℓ,% Pred.	8.23,119	6.39,130
VC	ℓ,% Pred.	6.79,128	4.75,120
FRC (Box)	ℓ,% Pred.	4.37,151	3.79,176
RV (Box)	ℓ,% Pred.	1.92,128	2.10,186
RV/TLC	% TLC	22	31
Trapped Air	ℓ	0.48	0.17
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	0.90,0.25	0.49,0.54
FIF	ℓ/S,% Pred.	7.62, 86	6.99,113
FEV_1	ℓ,% VC	4.63, 68	3.70, 78
ET-FEV_1	ℓ, ERV	1.34, 55	1.10, 65
FEF(25-75)	ℓ/S,% Pred.	3.26, 52	3.87, 87
$\dot{V}_{50}$	ℓ/S,% Pred.	3.80, 57	3.70, 81
$\dot{V}_{75}$	ℓ/S,% Pred.	1.40, 42	1.80, 79
DLCO	ml/min/mmHg,% Pred.	47.3,121	31.9,116
CV	% VC	12.4	12.2
CC	% TLC	27.2	37.2
Slope N_2 washout,	$\%N_2/\ell$	1.28	2.54
<u>After Bronchodilator</u> (Ventolin)			
VC	ℓ,% Pred.	6.85,129	4.45,112
FEV_1	ℓ,% VC	5.03, 73	4.13, 93
Raw $cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$		
$\dot{V}_{50}$	ℓ/S,% Pred.	4.20, 63	4.50,102
$\dot{V}_{75}$	ℓ/S,% Pred.	1.80, 54	1.90, 86

TABLE 11

SUMMARY OF PULMONARY FUNCTION DATA

ALL RESULTS EXPRESSED AS MEAN ± 1 SD

		All Responders	Non Responders
TLC (Box)	ℓ,% Pred.	6.19 ± 1.21,119 ± 11	7.19 ± 0.89,121 ± 12
TLC (He)	ℓ,% Pred.	5.58 ± 1.31,105 ± 12	6.35 ± 1.07,107 ± 13
VC	ℓ,% Pred.	4.15 ± 0.93,105 ± 9	4.99 ± 0.91,110 ± 12
FRC (Box)	ℓ,% Pred.	3.34 ± 0.88,151 ± 28	4.02 ± 0.46,162 ± 16
RV (Box)	ℓ,% Pred.	1.94 ± 0.62,160 ± 49	2.20 ± 0.27,166 ± 27
RV/TLC	% TLC	32 ± 7	31 ± 5
Trapped Air	ℓ	0.71 ± 0.31	0.81 ± 0.48
Raw$cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	.98 ± .47, .40 ± .22	.61 ± .42,.63 ± .41
FIF	ℓ/S,% Pred.	5.32 ± 0.98,101 ± 20	6.44 ± 1.63, 98 ± 17
FEV_1	ℓ,% VC	3.19 ± 0.64, 77 ± 7	3.70 ± 0.62, 76 ± 5
ET-FEV_1	ℓ, ERV	0.79 ± 0.27, 60 ± 15	1.25 ± 0.30, 69 ± 7
FEF(25-75)	ℓ/S,% Pred.	3.28 ± 1.02, 86 ± 30	3.77 ± 0.62, 83 ± 20
$\dot{V}_{50}$	ℓ/S,% Pred.	3.39 ± 1.16, 88 ± 32	3.90 ± 0.63, 84 ± 22
$\dot{V}_{75}$	ℓ/S,% Pred.	1.44 ± 0.65, 75 ± 37	1.56 ± 0.22, 68 ± 14
DLCO	ml/min/mmHg,% Pred.	27.6 ± 5.4, 105 ± 18	33.0 ± 8.8, 107 ± 14
CV	% VC	11.6 ± 6.3	10.0 ± 2.6
CC	% TLC	33.1 ± 6.0	30.5 ± 6.9
Slope N_2 washout,	$\%N_2/\ell$	2.09 ± 1.96	2.13 ± 0.98
<u>After Bronchodilator</u> (Ventolin)			
VC	ℓ,% Pred.	4.10 ± 0.94,105 ± 9	4.94 ± 0.96,105 ± 17
FEV_1	ℓ,% Pred.	3.37 ± 0.68, 84 ± 9	4.05 ± 0.61, 83 ± 6
Raw$cmH_2O/\ell/S$	SGaw$(cmH_2O/S)^{-1}$	.54 ± .23, .72 ± .43	
$\dot{V}_{50}$	ℓ/S,% Pred.	3.97 ± 1.36,102 ± 38	4.42 ± 0.92, 96 ± 22
$\dot{V}_{75}$	ℓ/S,% Pred.	1.78 ± 0.78, 88 ± 41	1.80 ± 0.30, 79 ± 23

TABLE 11,cont'd

SUMMARY OF PULMONARY FUNCTION DATA

ALL RESULTS EXPRESSED AS MEAN ± 1 SD

		Unequivocal Responders	Partial Responders
TLC (Box)	ℓ,% Pred.	6.04 ± 0.87,120 ± 10	6.46 ± 1.74,114 ± 16
TLC (He)	ℓ,% Pred.	5.36 ± 0.86,104 ± 10	6.02 ± 1.96,107 ± 17
VC	ℓ,% Pred.	4.03 ± 0.81,105 ± 8	4.40 ± 1.19,105 ± 12
FRC (Box)	ℓ,% Pred.	3.20 ± 0.62,151 ± 30	3.60 ± 1.29,151 ± 26
RV (Box)	ℓ,% Pred.	1.90 ± 0.60,161 ± 57	2.06 ± 0.67,156 ± 31
RV/TLC	% TLC	32 ± 8	32 ± 5
Trapped Air	ℓ	0.74 ± 0.24	0.65 ± 0.45
Raw$cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	1.05 ± .56, .43 ± .27	.86 ± .24, .36 ± .08
FIF	ℓ/S,% Pred.	5.42 ± 1.11,105 ± 20	5.14 ± 0.72, 94 ± 20
FEV_1	ℓ,% VC	3.12 ± 0.64, 78 ± 7	3.32 ± 0.69, 76 ± 7
ET-FEV_1	ℓ, ERV	0.74 ± 0.18, 62 ± 18	0.88 ± 0.39, 57 ± 9
FEF(25-75)	ℓ/S,% Pred.	3.27 ± 1.18, 86 ± 33	3.29 ± 0.70, 86 ± 27
$\dot{V}_{50}$	ℓ/S,% Pred.	3.46 ± 1.36, 90 ± 33	3.24 ± 0.70, 85 ± 32
$\dot{V}_{75}$	ℓ/S,% Pred.	1.53 ± 0.77, 80 ± 42	1.25 ± 0.22, 65 ± 24
DLCO	ml/min/mmHg,% Pred.	26.5 ± 4.4, 105 ± 20	29.8 ± 7.0, 105 ± 12
CV	% VC	11.6 ± 5.5	11.6 ± 9.0
CC	% TLC	32.2 ± 5.4	35.0 ± 7.6
Slope N_2 washout,	$\%N_2/\ell$	2.32 ± 2.33	1.57 ± 0.67
<u>After Bronchodilator</u> (Ventolin)			
VC	ℓ,% Pred.	3.96 ± 0.83,104 ± 8	4.38 ± 1.18,105 ± 11
FEV_1	ℓ,% VC	3.32 ± 0.71, 86 ± 10	3.48 ± 0.69, 81 ± 7
Raw$cmH_2O/\ell/S$	$SGaw(cmH_2O/S)^{-1}$	.53 ± .21, .77 ± .56	.56 ± .29, .63 ± .18
$\dot{V}_{50}$	ℓ/S,% Pred.	4.10 ± 1.61,105 ± 42	3.70 ± 0.71, 97 ± 33
$\dot{V}_{75}$	ℓ/S,% Pred.	1.96 ± 0.89, 94 ± 44	1.41 ± 0.32, 77 ± 36

APPENDIX D

TABLE 12

METHACHOLINE CHALLENGE TEST RESULTS

ID#	Control				Post-methacholine (% control)						Post-bronchodilator (% control)						PC_{20} FEV_1	Dose Units admin.
	FVC ℓ	FEV_1 ℓ	$\dot{V}_{50}$ ℓ/s	$\dot{V}_{75}$ ℓ/s	FVC	FEV_1	$\dot{V}_{50}$	$\dot{V}_{75}$	IsoVol $\dot{V}_{50}$	IsoVol $\dot{V}_{75}$	FVC	FEV_1	$\dot{V}_{50}$	$\dot{V}_{75}$	IsoVol $\dot{V}_{50}$	IsoVol $\dot{V}_{75}$		
1.	4.35	3.42	3.33	1.74	59	52	44	37	8	0	97	108	144	151	138	144	0.5	2.5
2.	3.56	2.62	2.98	0.62	88	81	62	64	51	33	110	98	68	71	94	108	25	217.5
3.	4.49	3.56	3.72	1.37	90	79	65	69	54	46	102	96	86	101	90	115	10	92.5
4.	2.98	2.69	5.09	1.87	76	69	49	40	23	0	99	98	94	96	95	91	5	40
5.	3.19	2.66	3.15	1.32	76	64	45	45	28	0	102	106	106	122	108	131	5	40
6.	4.84	4.00	5.18	1.90	73	60	38	41	18	0	102	102	100	96	100	106	25	217.5
7.	4.04	3.18	3.53	1.29	81	74	57	58	39	17	101	107	123	121	125	121	2	17.5
8.	3.32	2.79	3.69	1.72	63	65	99	67	20	0	111	117	162	135	171	190	10	92.5
9.	3.47	3.34	7.77	3.02	83	54	16	23	14	11	107	101	70	79	74	95	2	17.5
10.	3.10	2.43	3.23	0.74	86	79	58	68	41	28	104	106	120	109	132	169	10	92.5
11.	3.19	2.74	5.07	1.41	69	65	43	54	16	0	108	111	90	172	90	273	5	42.5
12.	2.73	2.25	2.66	1.05	88	75	64	53	45	24	102	104	98	143	103	150	5	42.5
13.	4.37	3.50	3.94	1.40	97	93	82	88	82	74	107	104	94	96	105	116	>25	217.5

*13. FEV_1 = 49% control at 1 minute after 25 mg/ml methacholine.

TABLE 12 cont'd...

METHACHOLINE CHALLENGE TEST RESULTS

ID#	Control				Post-methacholine (% control)						Post-bronchodilator (% control)						PC_{20} FEV_1	Dose Units admin.
	FVC ℓ	FEV_1 ℓ	$\dot{V}_{50}$ ℓ/s	$\dot{V}_{75}$ ℓ/s	FVC	FEV_1	$\dot{V}_{50}$	$\dot{V}_{75}$	IsoVol $\dot{V}_{50}$	IsoVol $\dot{V}_{75}$	FVC	FEV_1	$\dot{V}_{50}$	$\dot{V}_{75}$	IsoVol $\dot{V}_{50}$	IsoVol $\dot{V}_{75}$		
14.	4.54	3.44	3.49	1.05	95	87	75	83	73	75	114	111	109	119	148	200	>25	217.5
15.	5.18	3.71	3.29	1.50	94	91	84	76	80	46	99	102	108	75	113	77	>25	215
16.	2.72	2.37	4.16	1.65	104	99	79	62	85	75	99	102	111	95	108	88	>25	217.5
17.	3.45	3.12	5.55	2.05	98	89	69	57	64	64	101	98	91	91	94	117	>25	215
18.	2.78	2.39	3.31	1.24	88	86	72	78	55	34	99	99	95	92	96	92	>25	217.5
19.	3.79	2.81	2.82	1.73	99	101	102	70	102	65	105	109	114	72	117	76	>25	215
20.	4.84	4.14	4.67	2.33	96	90	65	55	64	50	104	105	141	109	145	123	>25	215
21.	4.20	3.55	4.94	1.94	99	97	90	93	89	84	102	101	90	95	90	95	>25	215
22.	5.24	4.31	6.89	1.86	99	95	71	82	73	88	93	97	102	117	100	69	>25	215
23.	4.91	3.88	3.79	1.72	93	89	79	83	72	63	101	103	106	106	106	106	>25	217.5
24.	3.56	3.06	5.05	1.61	97	94	91	79	88	67	101	101	100	100	100	109	>25	217.5
25.	6.37	4.64	4.13	1.62	94	88	67	72	64	56	103	106	115	100	120	119	>25	215
26.	4.36	3.68	4.50	1.81	100	93	81	92	81	82	102	106	120	128	130	138	>25	215

TABLE 13

SUMMARY OF METHACHOLINE CHALLENGE TEST RESULTS

(Mean ± 1SD)

Control				Post-methacholine (% control)						Post-bronchodilator (% control)						PC_{20} FEV_1	PD_{20} FEV_1
FVC ℓ	FEV_1 ℓ	$\dot{V}_{50}$ ℓ/s	$\dot{V}_{75}$ ℓ/s	FVC	FEV_1	$\dot{V}_{50}$	$\dot{V}_{75}$	IsoVol $\dot{V}_{50}$	IsoVol $\dot{V}_{75}$	FVC	FEV_1	$\dot{V}_{50}$	$\dot{V}_{75}$	IsoVol $\dot{V}_{50}$	IsoVol $\dot{V}_{75}$		
Unequivocal Responders																	
3.61	2.97	4.12	1.50	78	68	53	52	30	13	104	104	105	116	110	141	8.7	76
±0.67	±0.52	±1.44	±0.63	±10	±10	±20	±15	±16	±16	±4	±6	±28	±30	±27	±51	±8.2	±73
Partial Responders																	
3.84	3.09	3.96	1.48	96	90	77	74	73	62	103	103	101	95	111	115	>25	>215
±1.01	±0.58	±0.86	±0.35	±5	±6	±6	±12	±12	±18	±6	±5	±10	±14	±20	±45		
All Responders																	
3.68	3.01	4.06	1.50	84	76	61	59	44	29	104	104	104	109	110	132		
±0.78	±0.53	±1.25	±0.54	±12	±14	±20	±17	±25	±29	±5	±6	±23	±28	±24	±49		
Nonresponders																	
4.66	3.76	4.60	1.83	97	93	81	78	79	69	101	104	111	103	114	104	25	215
±0.90	±0.62	±1.17	±0.23	±3	±5	±13	±12	±12	±14	±4	±4	±15	±16	±18	±23		

APPENDIX E

TABLE 14

RVr/TLCr RESULTS

Subject #	Zone:	1	2	3	4	5	6	7	8	9	10
a) Initial											
1		.17	.16	.17	.14	.18	.18	.13	.14	.13	.16
2		.45	.29	.20	.23	.26	.42	.31	.23	.21	.25
3		.19	.13	.11	.09	.09	.19	.12	.10	.11	.09
4		.45	.34	.26	.22	.18	.42	.34	.27	.23	.19
5		.43	.31	.27	.18	.22	.39	.26	.22	.18	.20
6		.20	.18	.19	.20	.26	.22	.18	.19	.19	.22
7		.29	.28	.22	.24	.30	.28	.24	.19	.17	.25
8		.52	.36	.30	.24	.24	.55	.38	.27	.22	.24
9		.42	.38	.34	.26	.24	.43	.34	.30	.24	.24
10		.37	.31	.29	.26	.24	.43	.35	.29	.27	.25
11		.39	.32	.25	.21	.19	.33	.27	.24	.21	.18
12		.28	.25	.21	.21	.27	.30	.25	.21	.20	.23
Mean 1-12		.35	.28	.23	.21	.22	.34	.26	.22	.20	.21
SD 1-12		.12	.08	.06	.05	.05	.11	.08	.06	.04	.05
13		.35	.23	.18	.15	.13	.33	.22	.17	.14	.15
14		.48	.32	.27	.24	.23	.46	.31	.26	.24	.24
15		.34	.33	.27	.24	.21	.29	.25	.24	.25	.23
16		.29	.21	.19	.15	.19	.28	.21	.20	.18	.14
17		.29	.22	.19	.18	.17	.28	.19	.19	.22	.18
18		.35	.27	.25	.19	.30	.40	.27	.23	.17	.35
Mean 13-18		.35	.26	.23	.19	.21	.34	.24	.22	.20	.21
SD 13-18		.07	.05	.04	.04	.06	.07	.05	.04	.04	.08
Mean 1-18		.35	.27	.23	.20	.22	.34	.26	.22	.20	.21
SD 1-18		.10	.07	.06	.05	.06	.10	.07	.05	.04	.06

TABLE 14 cont'd...

RVr/TLCr RESULTS

Subject #	Zone:	1	2	3	4	5	6	7	8	9	10
a) Initial											
19		.30	.24	.19	.19	.16	.26	.20	.17	.19	.19
20		.29	.25	.19	.18	.15	.26	.19	.19	.18	.16
21		.34	.26	.18	.15	.20	.37	.26	.21	.16	.16
22		.40	.29	.21	.25	.19	.44	.30	.23	.21	.18
23		.39	.33	.27	.21	.15	.33	.28	.21	.17	.15
24		.41	.36	.32	.30	.31	.40	.35	.30	.29	.28
25		.27	.22	.23	.20	.19	.26	.21	.19	.19	.19
26		.36	.27	.23	.22	.22	.32	.24	.20	.22	.21
Mean 19-26		.34	.28	.23	.21	.20	.33	.25	.21	.21	.19
SD 19-26		.05	.05	.05	.05	.05	.07	.06	.04	.04	.04
Mean 1-26		.35	.27	.23	.20	.21	.34	.26	.22	.20	.20
SD 1-26		.09	.06	.05	.05	.05	.09	.07	.05	.04	.05
b) Post-methacholine											
1		.48	.47	.45	.34	.31	.45	.38	.36	.27	.29
2		.46	.44	.40	.37	.34	.44	.41	.35	.34	.36
3		.37	.41	.34	.28	.25	.30	.29	.26	.31	.27
4		.46	.34	.31	.29	.26	.50	.39	.34	.33	.24
5		.48	.36	.34	.25	.28	.44	.32	.24	.19	.23
6		.37	.31	.28	.25	.24	.36	.29	.25	.29	.31
7		.37	.28	.26	.26	.36	.31	.24	.24	.29	.35
8		.83	.60	.36	.33	.31	.99	.72	.41	.31	.39
9		.47	.42	.38	.34	.34	.46	.42	.39	.38	.36
10		.53	.38	.35	.32	.30	.52	.39	.34	.33	.30
11		.40	.31	.27	.33	.50	.43	.32	.26	.29	.43
12		.52	.36	.25	.26	.31	.41	.28	.21	.23	.26
Mean 1-12		.48	.39	.33	.30	.32	.47	.37	.30	.30	.32
SD 1-12		.12	.09	.06	.04	.07	.18	.12	.07	.05	.06

TABLE 14 cont'd...

RVr/TLCr RESULTS

Subject #	Zone:	1	2	3	4	5	6	7	8	9	10
b) Post-methacholine											
	13	.40	.30	.20	.16	.18	.39	.26	.19	.18	.17
	14	.47	.39	.34	.31	.27	.51	.41	.38	.37	.34
	15	.43	.38	.31	.24	.20	.37	.28	.24	.21	.22
	16	.40	.29	.25	.25	.30	.43	.31	.32	.30	.38
	17	.29	.23	.22	.22	.22	.28	.21	.20	.22	.24
	18	.36	.30	.25	.17	.24	.46	.27	.21	.19	.23
Mean	13-18	.39	.31	.26	.22	.23	.41	.29	.26	.25	.26
SD	13-18	.06	.06	.05	.06	.04	.08	.07	.08	.07	.08
Mean	1-18	.45	.37	.31	.28	.29	.45	.34	.29	.28	.30
SD	1-18	.11	.09	.07	.06	.07	.15	.11	.07	.06	.07
	19	.27	.23	.18	.18	.16	.22	.20	.16	.15	.18
	20	.27	.22	.16	.15	.14	.28	.20	.17	.16	.14
	21	.31	.26	.18	.16	.19	.36	.25	.19	.18	.18
	22	.37	.26	.27	.20	.19	.32	.26	.22	.18	.19
	23	.38	.35	.30	.24	.22	.38	.29	.26	.20	.20
	24	.42	.33	.29	.29	.32	.42	.35	.29	.26	.32
	25	.28	.25	.23	.17	.19	.20	.24	.22	.20	.19
	26	.32	.28	.25	.21	.18	.30	.24	.21	.21	.19
Mean	19-26	.33	.27	.23	.20	.20	.32	.25	.21	.19	.20
SD	19-26	.06	.05	.05	.05	.05	.06	.05	.04	.04	.05
c) Post-Ventolin											
	1	.20	.18	.18	.18	.18	.21	.18	.16	.14	.16
	2	.53	.31	.22	.23	.20	.46	.34	.24	.18	.19
	3	.30	.22	.20	.19	.15	.29	.25	.18	.12	.10
	4	.48	.38	.28	.22	.20	.45	.36	.26	.22	.20
	5	.39	.32	.30	.21	.21	.36	.28	.20	.16	.21

TABLE 14 cont'd...

RVr/TLCr RESULTS

Subject #	Zone:	1	2	3	4	5	6	7	8	9	10
c) Post-Ventolin											
6		.21	.19	.18	.18	.21	.21	.18	.16	.18	.20
7		.34	.30	.24	.19	.20	.28	.23	.20	.20	.21
8		.71	.50	.36	.28	.29	.65	.48	.32	.30	.29
9		.46	.34	.27	.20	.20	.43	.30	.22	.20	.22
10		.44	.34	.34	.31	.30	.53	.40	.33	.34	.30
11		.44	.35	.28	.23	.22	.35	.29	.25	.24	.20
12		.40	.30	.25	.21	.23	.41	.28	.21	.19	.20
Mean 1-12		.41	.31	.26	.22	.21	.39	.30	.23	.21	.21
SD 1-12		.14	.09	.06	.04	.04	.13	.09	.06	.06	.05
13		.40	.27	.23	.18	.14	.38	.26	.20	.18	.15
14		.46	.36	.33	.30	.22	.48	.39	.32	.30	.26
15		.37	.36	.28	.23	.21	.31	.27	.22	.21	.20
16		.34	.25	.24	.21	.20	.32	.21	.21	.19	.19
17		.29	.24	.20	.19	.20	.30	.23	.21	.21	.22
18		.27	.25	.20	.14	.20	.31	.23	.18	.16	.20
Mean 13-18		.36	.29	.24	.21	.19	.35	.26	.22	.21	.20
SD 13-18		.07	.06	.05	.06	.03	.07	.06	.05	.05	.03
Mean 1-18		.39	.30	.25	.22	.21	.37	.29	.23	.21	.20
SD 1-18		.12	.08	.05	.04	.04	.11	.08	.05	.06	.05

TABLE 14 cont'd...

RVr/TLCr RESULTS

Subject #	Zone:	1	2	3	4	5	6	7	8	9	10
c) Post-Ventolin											
19		.27	.23	.19	.18	.14	.23	.20	.18	.17	.19
20		.32	.23	.19	.19	.17	.29	.19	.19	.17	.15
21		.34	.24	.19	.17	.17	.34	.25	.18	.21	.15
22		.25	.20	.17	.15	.13	.23	.17	.14	.13	.13
23		.47	.43	.38	.29	.20	.43	.37	.32	.26	.20
24		.40	.33	.32	.32	.32	.42	.37	.32	.29	.27
25		.25	.23	.20	.17	.16	.25	.22	.21	.19	.19
26		.29	.26	.24	.23	.21	.30	.23	.20	.20	.19
Mean 19-26		.32	.27	.24	.21	.19	.31	.25	.22	.20	.18
SD 19-26		.08	.07	.07	.06	.06	.08	.08	.06	.05	.04

TABLE 15

SUMMARY OF RVr/TLCr DATA

(Mean ± 1SD)

1	2	3	4	5	6	7	8	9	10
All Responders (n=18)									
Initial									
.35 ± .10	.27 ± .07	.23 ± .06	.20 ± .05	.22 ± .06	.34 ± .10	.26 ± .07	.22 ± .05	.20 ± .04	.21 ± .06
Post-methacholine									
.45 ± .11	.37 ± 09	.31 ± .07	.28 ± .06	.29 ± .07	.45 ± .15	.34 ± .01	.29 ± .07	.28 ± .06	.30 ± .07
Post-Ventolin									
.39 ± .12	.30 ± .08	.25 ± .05	.22 ± .04	.21 ± .04	.37 ± .11	.29 ± .08	.23 ± .05	.21 ± .06	.20 ± .05
Unequivocal Responders (n=12)									
Initial									
.35 ± .12	.28 ± .08	.23 ± .06	.21 ± .05	.22 ± .05	.34 ± .11	.26 ± .08	.22 ± .06	.20 ± .04	.21 ± .05
Post-methacholine									
.48 ± .12	.39 ± .09	.33 ± .06	.30 ± .04	.32 ± .07	.47 ± .18	.37 ± .12	.30 ± .07	.30 ± .05	.32 ± .06
Post-Ventolin									
.41 ± .14	.31 ± .09	.26 ± .06	.22 ± .04	.21 ± .04	.39 ± .13	.30 ± .09	.23 ± .06	.21 ± .06	.21 ± .05

TABLE 15 cont'd...

SUMMARY OF RVr/TLCr DATA

(Mean ± 1SD)

1	2	3	4	5	6	7	8	9	10
"Partial" Responders (n=6)									
Initial									
.35 ± .07	.26 ± .05	.23 ± .04	.19 ± .04	.21 ± .06	.34 ± .07	.24 ± .05	.22 ± .04	.20 ± .04	.21 ± .08
Post-methacholine									
.39 ± .06	.31 ± .06	.26 ± .05	.22 ± .06	.23 ± .04	.41 ± .08	.29 ± .07	.26 ± .08	.25 ± .07	.26 ± .08
Post-Ventolin									
.36 ± .07	.29 ± .06	.24 ± .05	.21 ± .06	.19 ± .03	.35 ± .07	.26 ± .06	.22 ± .05	.21 ± .05	.20 ± .03
Nonresponders (n=8)									
Initial									
.34 ± .05	.28 ± .05	.23 ± .05	.21 ± .05	.20 ± .05	.33 ± .07	.25 ± .06	.21 ± .04	.21 ± .04	.19 ± .04
Post-methacholine									
.33 ± .06	.27 ± .05	.23 ± .05	.20 ± .05	.20 ± .05	.32 ± .06	.25 ± .05	.21 ± .04	.19 ± .04	.20 ± .05
Post-Ventolin									
.32 ± .08	.27 ± .07	.24 ± .07	.21 ± .06	.19 ± .06	.31 ± .08	.25 ± .08	.22 ± .06	.20 ± .05	.18 ± .04
All Subjects (n=26)									
Initial									
.35 ± .09	.27 ± .06	.23 ± .05	.20 ± .05	.21 ± .05	.34 ± .09	.26 ± .07	.22 ± .05	.20 ± .04	.20 ± .05

TABLE 16

PROBABILITY (P) VALUES BETWEEN RVr/TLCr MEASUREMENTS SPECIFIED (Paired t test)

NS = not significant at p<.05)

	1	2	3	4	5	6	7	8	9	10
All Responders (Subjects 1-18)										
Initial vs post-methacholine	.008	.001	<.001	<.001	.002	.021	.010	.002	<.001	<.001
Post-methacholine vs post-Ventolin	NS	.029	.010	.002	<.001	NS	NS	.006	<.001	<.001
Initial vs post-Ventolin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Unequivocal Responders (Subjects 1-12)										
Initial vs post-methacholine	.014	.003	<.001	<.001	<.001	NS	.022	.004	<.001	<.001
Post-methacholine vs post-Ventolin	NS	.032	.005	<.001	<.001	NS	NS	.008	<.001	<.001
Initial vs post-Ventolin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
"Partial" Responders (Subjects 13-18)										
Initial vs post-methacholine	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Post-methacholine vs post-Ventolin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Initial vs post-Ventolin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Nonresponders (Subjects 19-26)										
Initial vs post-methacholine	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Post-methacholine vs post-Ventolin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Initial vs post-Ventolin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

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